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CLINICAL RESEARCH **Interventional Cardiology**

Aspirin and Clopidogrel Drug Response in Patients Undergoing Percutaneous Coronary Intervention

The Role of Dual Drug Resistance

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OBJECTIVES We sought to evaluate the response to clopidogrel among aspirin-resistant versus aspirin-sensitive patients undergoing percutaneous coronary intervention (PCI).

BACKGROUND Wide variability has been reported in response to aspirin and clopidogrel. There are limited data on the simultaneous responses to both drugs.

METHODS Elective PCI patients (n = 150) who received aspirin for ≥1 week but not clopidogrel were included. All patients received bivalirudin during PCI. Blood samples were drawn at baseline and 20 to 24 h after a 300-mg clopidogrel dose. Aspirin resistance was defined by ≥2 of 3 criteria: rapid platelet function analyzer-ASA score ≥550, 5 μmol/l adenosine diphosphate (ADP)-induced aggregation ≥70%, and 0.5 mg/ml arachidonic acid-induced aggregation ≥20%. Clopidogrel resistance was defined as baseline minus post-treatment aggregation ≤10% in response to 5 and 20 μmol/l ADP.

RESULTS Nineteen (12.7%) patients were resistant to aspirin and 36 (24%) to clopidogrel. Nine (47.4%) of the aspirin-resistant patients were also clopidogrel resistant. Aspirin-resistant patients were more likely to be women and have diabetes than were aspirin-sensitive patients. They also had lower response to clopidogrel, assessed by platelet aggregation and activation markers (flow cytometry-determined PAC-1 binding and P-selectin expression). Elevation of creatine kinase-myocardial band after stenting occurred more frequently in aspirin-resistant versus aspirin-sensitive patients (38.9% vs. 18.3%; p = 0.04) and in clopidogrel-resistant versus clopidogrel-sensitive patients (32.4% vs. 17.3%; p = 0.06).

CONCLUSIONS Aspirin-resistant patients as a group have reduced response to clopidogrel. Furthermore, we have identified a unique group of dual drug-resistant patients who may be at increased risk for thrombotic complications after PCI. (J Am Coll Cardiol 2006;47:27–33) © 2006 by the American College of Cardiology Foundation

Aspirin and clopidogrel have become standard therapy in patients undergoing percutaneous coronary intervention (PCI) with stenting. However, there is considerable heterogeneity in the responses of individual patients to each of these drugs (1–4). Previous studies have estimated that adequate antiplatelet effects are not achieved in 5% to 45% of patients taking aspirin and 4% to 30% of patients taking clopidogrel (1,3–8).

Resistance to the antiplatelet effects of aspirin has been associated with adverse clinical outcomes (2,5) and with an increase in markers of myonecrosis following PCI (9). It has been proposed that aspirin-resistant patients be treated routinely with alternative antiplatelet drugs, mainly clopidogrel. However, it is not clear whether the response to clopidogrel is similar in aspirin-resistant and aspirin-sensitive patients. Platelets from aspirin-resistant patients appear to have increased sensitivity to agonists such as adenosine diphosphate (ADP) and collagen (10,11). Furthermore, aspirin resistance has been associated with platelet hyperreactivity (10,12). These hyperreactive platelets may also be less responsive to inhibition by other antiplatelet drugs such as clopidogrel.

There are limited data regarding the simultaneous responses to both aspirin and clopidogrel. Lepantalo et al. (13) recently reported that among 50 patients undergoing PCI, 5 (10%) were "poor responders" to both aspirin and clopidogrel. Although this study is limited by the small number of patients, it suggests that a subgroup of patients may have low response to both drugs. Our aim, therefore, was to evaluate prospectively the response to clopidogrel among aspirin-resistant versus aspirin-sensitive patients, and to characterize factors that affect the responses to either drug in patients undergoing elective PCI.

METHODS

Patients. We enrolled patients scheduled for elective PCI between November 2003 and February 2005. All patients had received aspirin 81 to 325 mg daily for ≥1 week before PCI and had not received a thienopyridine or glycoprotein (GP) IIb/IIIa inhibitor in the week prior to enrollment. Patients were enrolled if they were planned to receive

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Abbreviations and Acronyms

AA	= arachidonic acid
ADP	= adenosine diphosphate
ARU	= aspirin reaction units
CK-MB	= creatine kinase-myocardial band
GP	= glycoprotein
MFI	= mean fluorescence intensity
MI	= myocardial infarction
PCI	= percutaneous coronary intervention
RPFA-ASA	= rapid platelet function assay-aspirin

bivalirudin rather than heparin and a GP IIb/IIIa inhibitor during PCI, because bivalirudin does not affect ADP-induced platelet aggregation (14). Exclusion criteria were acute myocardial infarction (MI) within one week, any contraindications to aspirin, clopidogrel, or bivalirudin, thrombocytopenia ($<100 \times 10^3$ cells/mm^3), anemia (hemoglobin <10 g/dl), or renal failure (creatinine >2.5 mg/dl).

This study was approved by the Investigational Review Board of the Baylor College of Medicine; all patients gave informed consent. Our aim was to enroll 150 patients. One hundred sixty patients were initially enrolled. Ten patients receiving GP IIb/IIIa inhibitors during PCI were withdrawn from the study, leaving 150. All patients underwent coronary stent implantation.

Medications. Immediately following PCI all patients received 300 mg clopidogrel and 325 mg oral aspirin in the catheterization laboratory under direct supervision, followed by 75 mg clopidogrel and 325 mg aspirin daily thereafter. During PCI all patients received a standard course of intravenous bivalirudin bolus 0.75 mg/kg followed by an infusion of 1.75 mg/kg/h until PCI completion.

Blood sampling. Two blood samples were collected in tubes containing 3.2% citrate. The tubes were filled to capacity and then gently mixed. The first (baseline) blood sample was obtained in the catheterization laboratory, prior to PCI and clopidogrel loading, from a 6- to 7-F arterial sheath. The second sample was obtained from an antecubital vein, using a 21-gauge needle, 20 to 24 h after PCI. Blood samples were processed within 1 h of collection.

Platelet aggregation. Turbidimetric platelet aggregation was performed in platelet-rich plasma with a platelet count adjusted to 250×10^3/mm^3. Platelets were stimulated with 0.5 mg/ml (1.6 mmol/l) arachidonic acid (AA) and with 5 and 20 μmol/l ADP. Aggregation was performed with a BioData PAP-4 platelet aggregometer (BioData, Horsham, Pennsylvania). The extent of aggregation was defined as the maximal light transmission ≤6 min after addition of the agonist, with platelet-poor plasma used as reference.

Platelet activation. Platelet activation was determined by assessing platelet surface expression of activated GP IIb/IIIa receptors and P-selectin in response to ADP stimulation, using flow cytometry as previously described (15). Briefly, GP IIb/IIIa activation was assessed using a fluorescein isothiocyanate-conjugated PAC-1 antibody (Becton Dickinson, San Jose, California), and P-selectin expression was determined using an R-phycoerythrin-conjugated anti-CD62P antibody (BD Pharmingen, San Jose, California). Citrated whole blood was diluted with Tyrode's buffer and stimulated for 5 min with 10 μmol/l ADP (final concentration). After adding the corresponding antibody and incubating for 20 min, the mixture was fixated with phosphate-buffered saline containing 1% paraformaldehyde. Samples were analyzed with a Coulter Epics XL MCL flow cytometer (Beckman-Coulter, Miami, Florida). Non-stimulated samples served as negative controls. Both PAC-1 binding and P-selectin were expressed as log mean fluorescence intensity (MFI) and as percentage change in MFI from baseline to the post-PCI sample.

Rapid platelet function assay-aspirin (RPFA-ASA). A point-of-care system (VerifyNow; Accumetrics, San Diego, California), that uses cartridges containing fibrinogen-coated beads and platelet agonists, RPFA-ASA measures platelet agglutination in response to metallic cations and propyl gallate, which activate the cyclooxygenase-1 pathway. (The RPFA-ASA system we used differs from the currently available assay, which employs AA as the agonist.) Results are expressed as aspirin reaction units (ARU). An ARU ≥550 indicates that aspirin-induced platelet dysfunction has not been detected (9).

Markers of myonecrosis. Creatine kinase-myocardial band (CK-MB) levels were measured from frozen plasma samples taken 20 to 24 h after PCI, using a sandwich immunoassay (Advia Centaur CKMB assay; Bayer HealthCare, Tarrytown, New York). Creatine-MB levels were available for 144 patients. In all patients who had elevated levels of CK-MB after PCI, normal CK-MB levels at baseline were confirmed using the same assay. The upper limit of normal for CK-MB is 5.0 ng/ml.

Definitions. Clopidogrel resistance was defined as an absolute difference between baseline and post-treatment aggregation ≤10% in response to both 5 and 20 μmol/l ADP (3,8). High post-clopidogrel platelet aggregation was defined as >75th percentile aggregation in response to 5 and 20 μmol/l ADP (16). The definition of aspirin resistance has been less uniform (17). We employed a primary definition that incorporated previously used criteria (5,6,9) and required the presence of at least two of the following three: 1) 0.5 mg/ml AA-induced platelet aggregation ≥20%; 2) 5 μmol/l ADP-induced platelet aggregation ≥70%; and 3) RPFA-ASA ARU ≥550. To enable comparison with previous studies, alternate analyses of the association between aspirin and clopidogrel response were performed using two additional definitions: 1) criteria 1 + 2 (5); and 2) criterion 3 (9). Aspirin resistance was determined using the baseline blood samples.

Sample size and statistical analysis. The sample size was predetermined based on logistic regression power analysis with a clopidogrel resistance rate of 30% used as the end point (3). Logistic regression of response to clopidogrel with

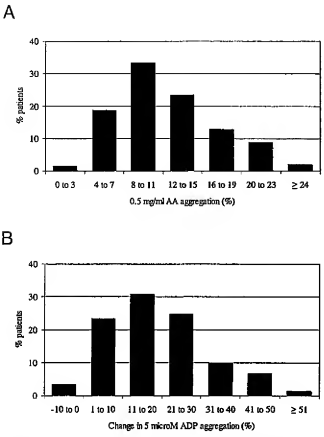

Figure 1. Distribution of the response to **(A)** aspirin (assessed by 0.5 mg/ml arachidonic acid [AA]-induced platelet aggregation) and **(B)** clopidogrel (evaluated by change in 5 μmol/l adenosine diphosphate [ADP]-induced aggregation) from baseline to post-treatment. Both distributions were normal ($p = 0.0003$ and $p = 0.01$, respectively).

a sample size of 150 observations achieves 80% power at 0.05 significance level to detect a change of 20% between the two study groups (aspirin resistant vs. aspirin sensitive).

Continuous variables are presented as mean values ± SD. Comparisons between continuous variables were performed using unpaired Student *t* tests, because they were normally distributed (demonstrated by the Shapiro-Wilk test) (Fig. 1). Comparisons between categorical variables were performed using Fisher exact tests if any subgroups consisted of five or fewer items; otherwise, chi-square tests were used. The response to clopidogrel, expressed as change in platelet aggregation or activation markers from baseline to post-treatment, was compared among aspirin-resistant versus sensitive patients using unpaired Student *t* tests. Two further analyses were performed. The response to clopidogrel was compared among tertiles of AA-induced aggregation using analysis of variance (ANOVA). In addition, the percentages of patients with high post-clopidogrel aggregation were compared with aspirin-resistant versus aspirin-sensitive patients. Analyses were performed using SPSS version 11 statistical software (SPSS Inc., Chicago, Illinois); statistical significance was set at $p < 0.05$.

RESULTS

Clopidogrel and aspirin resistance rates. Thirty-six patients (24%) met the definition of clopidogrel resistance. Aspirin resistance was observed in 19 patients (12.7%) using the primary definition (≥2 of the three criteria), 14 patients (9.3%) using the definition of AA-induced aggregation ≥20% and 5 μmol/l ADP-induced aggregation ≥70%, and 23 patients (15.3%) with the definition of RPFA-ASA ARU ≥550 (Table 1). Regardless of which aspirin resistance definition was used, about 50% of patients who were aspirin resistant were also resistant to clopidogrel, and about 20% of aspirin-sensitive patients were clopidogrel resistant ($p \leq 0.02$) (Table 1). All subsequent comparisons between aspirin-resistant and aspirin-sensitive patients were performed using the primary definition.

To assess the role of prior medication compliance on aspirin resistance we compared AA-induced aggregation before and 20 to 24 h after the witnessed dose of aspirin. There were no significant differences in AA-induced aggregation between the two time points among aspirin-resistant patients (pre-PCI 20.2 ± 4.5% vs. post-PCI 18.8 ± 2.9%; $p = 0.2$) or among aspirin-sensitive patients (pre-PCI 10.5 ± 4.7% vs. post-PCI 10 ± 3.7%; $p = 0.3$).

Patient and procedural characteristics. Compared with aspirin-sensitive patients, aspirin-resistant patients were more likely to be women and to have diabetes (Table 2). Of the 47 women, 11 (23.4%) were aspirin resistant compared with only 8 (7.8%) of the 103 men ($p = 0.01$). Aspirin-resistant patients also had lower hemoglobin levels than aspirin-sensitive patients. There were no differences in patient characteristics between clopidogrel-resistant and clopidogrel-sensitive patients (Table 2). We also compared the characteristics of dual drug-resistant patients (resistant to both aspirin and clopidogrel; $n = 9$) to those of dual

Table 1. Rates of Clopidogrel Resistance in Aspirin-Resistant Versus Aspirin-Sensitive Patients

Aspirin Resistance Definition	ASA-Resistant Patients (n)	Clopidogrel Resistance Among ASA-Resistant Patients	ASA-Sensitive Patients (n)	Clopidogrel Resistance Among ASA-Sensitive Patients	p Value
At least 2 of the 3 criteria	19	9 (47.4%)	131	27 (20.6%)	0.01
AA aggregation ≥20% and ADP aggregation ≥70%*	14	7 (50%)	136	29 (21.3%)	0.02
RPFA-ASA ARU ≥550	23	11 (47.8%)	127	25 (19.7%)	0.01

*0.5 mg/ml arachidonic acid-induced aggregation ≥20% and 5 μmol/l ADP-induced aggregation ≥70%.
ADP = adenosine diphosphate; ASA = aspirin; RPFA-ASA ARU = rapid platelet function assay-ASA expressed in aspirin reaction units.

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Table 2. Baseline Clinical Characteristics, Laboratory Data, and Medications

	Aspirin Resistant (n = 19)	Aspirin Sensitive (n = 131)	Clopidogrel Resistant (n = 36)	Clopidogrel Sensitive (n = 114)
Age (yrs)	67.2 ± 10.6	65.3 ± 10.6	64.3 ± 10.1	65.9 ± 11.3
Women	11 (57.9%)*	36 (27.5%)*	15 (41.7%)	32 (28.1%)
BMI (kg/m^2)	30.9 ± 7.2	29.8 ± 5	31.3 ± 6	29.4 ± 5
Diabetes	10 (52.6%)†	38 (29%)†	12 (33.3%)	36 (31.6%)
Hypertension	16 (84.2%)	110 (84%)	30 (83.3%)	96 (84.2%)
Hyperlipidemia	16 (84.2%)	91 (69.5%)	26 (72.2%)	81 (71.1%)
Smoking	7 (36.8%)	42 (32.1%)	11 (30.6%)	38 (33.3%)
Prior MI	3 (15.8%)	26 (19.8%)	9 (25%)	20 (17.5%)
Prior CABG	3 (15.8%)	28 (21.4%)	8 (22.2%)	23 (20.2%)
Laboratory data				
Hemoglobin (g/dl)	12.7 ± 1.3*	13.9 ± 1.6*	13.6 ± 1.8	13.8 ± 1.5
WBC (10^3/mm^3)	8.7 ± 2.6	7.4 ± 2.4	8.2 ± 2.5	7.3 ± 2.4
Platelets (10^3/mm^3)	236.9 ± 71	204.8 ± 62	218.4 ± 64	204.9 ± 62.8
Mean platelet volume (fl)	9.8 ± 1.6	9.4 ± 1.2	9.9 ± 1.5	9.3 ± 1.4
Creatinine (mg/dl)	1.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
Baseline medications				
Aspirin 81 mg	10 (52.6%)	50 (38.2%)	15 (41.7%)	45 (39.5%)
Aspirin 325 mg	9 (47.4%)	81 (61.8%)	21 (58.3%)	69 (60.5%)
Statins	16 (84.2%)	92 (70.2%)	28 (77.8%)	80 (70.2%)
Beta-blockers	14 (73.7%)	72 (55%)	25 (69.4%)	61 (53.5%)
ACEI/ARB	6 (31.6%)	50 (38.2%)	9 (25%)	47 (41.2%)
CCB	3 (15.8%)	26 (19.8%)	5 (13.9%)	24 (21.1%)

*p ≤ 0.01; †p ≤ 0.05; for aspirin resistance vs. aspirin sensitivity.
ACEI = angiotensin-converting enzyme inhibitors; ARB = angiotensin receptor blockers; BMI = body mass index; CABG = coronary artery bypass graft; CCB = calcium channel blockers; MI = myocardial infarction; Smoking = current or former; WBC = white blood cells.

drug-sensitive patients (n = 104). Dual drug-resistant patients were more likely to be women (67.7% vs. 26.9%; p = 0.02) and had higher mean body mass index (33.8 ± 7.9 kg/m^2 vs. 29.7 ± 5 kg/m^2; p = 0.03). Among the whole study cohort, 60 (40%) patients were treated with 81 mg aspirin at the time of enrollment and 90 (60%) were treated with 325 mg. Ten (16.7%) of the 60 patients receiving 81 mg were aspirin resistant, compared with 9 (10%) of the 90 patients receiving 325 mg (p = 0.25).

There were no differences in indications for the PCI or procedural characteristics between aspirin-resistant and aspirin-sensitive patients or between clopidogrel-resistant and clopidogrel-sensitive patients (Table 3).

Response to clopidogrel among aspirin-resistant versus -sensitive patients. Aspirin-resistant patients had a significantly lower degree of reduction of platelet aggregation in response to 5 and 20 μmol/l ADP after clopidogrel (Table 4). They also displayed less inhibition of PAC-1 binding and a lower degree of reduction in P-selectin expression after clopidogrel treatment (Table 4).

The percentage of patients with high post-clopidogrel ADP-induced aggregation (>75th percentile) was higher among aspirin-resistant than aspirin-sensitive patients (5 μmol/l ADP: 78.9% vs. 18.3%, p = 0.001; 20 μmol/l ADP: 73.4% vs. 19.1%, p = 0.001). Furthermore, comparison of the change in ADP-induced aggregation among tertiles

Table 3. Indications for Percutaneous Coronary Intervention and Procedural Characteristics

	Aspirin Resistant (n = 19)	Aspirin Sensitive (n = 131)	Clopidogrel Resistant (n = 36)	Clopidogrel Sensitive (n = 114)
Indication				
Stable angina	10 (52.6%)	55 (42%)	19 (52.8%)	46 (40.4%)
Unstable angina	4 (21.1%)	34 (26%)	8 (22.2%)	30 (26.3%)
NSTEMI >1 week	3 (15.8%)	11 (8.4%)	2 (5.6%)	12 (10.5%)
(+) Stress test	4 (21.1%)	46 (35.7%)	12 (33.3%)	38 (33.3%)
Procedural characteristics				
Total stent length (mm)	22.6 ± 9.3	21.2 ± 9.9	23.5 ± 11	20.7 ± 9.4
Minimal stent diameter (mm)	3.0 ± 0.6	3.0 ± 0.4	3.0 ± 0.5	3.0 ± 0.4
No. of stents/patient	1.4 ± 0.6	1.3 ± 0.6	1.5 ± 0.7	1.3 ± 0.5
Drug-eluting stents	15 (78.9%)	117 (89.3%)	31 (86.1%)	100 (87.7%)
Bare-metal stents	4 (21.1%)	14 (10.7%)	5 (13.9%)	14 (12.3%)

NSTEMI = non-ST-segment elevation myocardial infarction; (+) Stress test = positive stress test.

Table 4. Response to Clopidogrel Among Aspirin-Resistant Versus Aspirin-Sensitive Patients

	Aspirin Resistant (n = 19)	Aspirin Sensitive (n = 131)	p Value
Absolute change in 20 μmol/l ADP aggregation (%)	8.3 ± 7.3	15.0 ± 10.2	0.001
Absolute change in 5 μmol/l ADP aggregation (%)	13.8 ± 9.7	20.6 ± 12.5	0.01
Absolute change in PAC-1 binding (MFI)	0.8 ± 1.5	1.7 ± 1.2	0.01
Absolute change in P-selectin expression (MFI)	4.1 ± 3.4	5.6 ± 3.5	0.09
Relative change in PAC-1 binding (%)	14.6 ± 25.6	35 ± 24.1	0.002
Relative change in P-selectin expression (%)	25.9 ± 23.8	40.4 ± 21.8	0.02

Absolute change = absolute difference between baseline and post-treatment aggregation; relative change = percentage decrease from baseline.
ADP = adenosine diphosphate; MFI = mean fluorescence intensity.

of AA-induced aggregation revealed a significant difference between the tertiles (5 μmol/l ADP, p = 0.006; 20 μmol/l ADP, p = 0.0001) (Fig. 2). Patients in the highest tertile (reflecting lower response to aspirin) had the least reduction in ADP-induced aggregation after clopidogrel treatment.

Comparison of baseline platelet reactivity showed a trend toward higher baseline P-selectin levels among aspirin-resistant versus aspirin-sensitive patients (15.6 ± 5.1 MFI vs. 13.8 ± 4.2 MFI; p = 0.1).

Markers of myonecrosis. Levels of CK-MB post-PCI were available for 144 patients. Thirty (20.8%) of the 144 patients had CK-MB levels above the upper limit of normal (Fig. 3). Elevation of CK-MB occurred more frequently in aspirin-resistant than in aspirin-sensitive patients (38.9% vs. 18.3%; p = 0.04) and in dual drug-resistant than in dual drug-sensitive patients (44.4% vs. 15.8%; p = 0.05). There was also a trend toward more frequent CK-MB elevations among clopidogrel-resistant versus clopidogrel-sensitive patients (32.4% vs. 17.3%; p = 0.06).

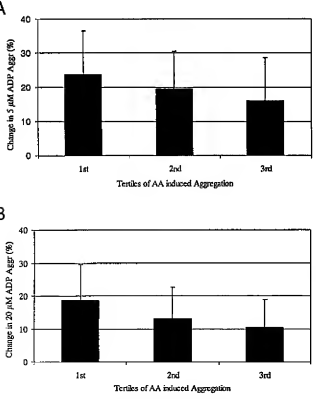

Figure 2. Response to clopidogrel among the three tertiles of 0.5 mg/ml arachidonic acid (AA)-induced aggregation (reflecting response to aspirin). Aggregation in response to (**A**) 5 μmol/l and (**B**) 20 μmol/l adenosine diphosphate (ADP) (p = 0.006 and p = 0.0001, respectively, for difference between tertiles).

DISCUSSION

This is the first study to characterize the response to clopidogrel among aspirin-resistant compared with aspirin-sensitive patients. It is also the first study of antiplatelet drug response to be performed in the presence of a direct thrombin inhibitor rather than unfractionated heparin, in order to avoid the confounding effects of heparin on platelet activity. We observed aspirin resistance in 9% to 15% of patients, depending on the definition used, and clopidogrel resistance in 24%. About one-half of the aspirin-resistant patients were also resistant to the effects of clopidogrel. Furthermore, we have shown that aspirin-resistant patients as a group display a lower inhibitory response to clopidogrel than aspirin-sensitive patients.

Clinical factors associated with drug resistance. Our secondary objective was to identify clinical factors associated with low response to aspirin or clopidogrel. Aspirin-resistant and dual drug-resistant patients were more likely to be women compared with aspirin-sensitive and dual drug-sensitive patients. This finding is in accordance with the studies of Gum et al. (5) and Chen et al. (9), who also found a higher proportion of women among aspirin-resistant

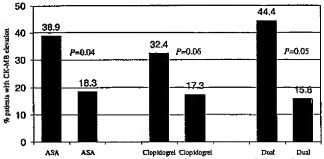

Figure 3. Incidence of creatinine kinase-myocardial band (CK-MB) elevation above the upper limit of normal in aspirin (ASA)-resistant versus aspirin-sensitive patients, clopidogrel-resistant versus clopidogrel-sensitive patients, and dual drug-resistant versus dual drug-sensitive patients.

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patients. The greater proportion of women may explain the lower hemoglobin level we observed among aspirin-resistant compared with aspirin-sensitive patients. The consistently higher rates of aspirin resistance among women may also account in part for the recently reported failure of aspirin to reduce the risk of a first MI in women, in contrast to its beneficial primary prevention effects in men (18).

An additional clinical factor we found to be associated with aspirin resistance is diabetes. Platelets from individuals with type 2 diabetes have been shown to have a reduced response to aspirin (19). Furthermore, obesity and insulin resistance have been associated with impaired platelet-inhibitory effects of aspirin in non-diabetic patients (20). This association may explain the significantly elevated BMI we observed in the dual drug-resistant group in our study. Lepantalo et al. (13) reported that low response to clopidogrel was associated with high levels of glycosylated hemoglobin and C-peptide. Therefore, insulin resistance may be associated with reduced response to both drugs.

Possible mechanisms for dual drug resistance. There are several plausible explanations for our findings of lower response to clopidogrel among aspirin-resistant patients as a group. The most likely mechanism is a global increase in platelet reactivity. Platelets from aspirin-resistant patients appear to have increased sensitivity to ADP-induced GP IIb/IIIa activation (10) as well as to low concentrations of collagen (11). Furthermore, patients with diabetes, who comprised more than half of the aspirin-resistant group, have been shown to have a higher proportion of platelets expressing P-selectin and activated GP IIb/IIIa receptors than non-diabetic patients (21,22). Although we observed only a trend toward higher baseline P-selectin expression in aspirin-resistant patients, if indeed these patients have hyper-reactive platelets they may be less sensitive to inhibition by clopidogrel.

Two other mechanisms are also possible. First, increased platelet turnover in aspirin-resistant patients may lead to the release of young platelets still able to form thromboxane A2 through non-cyclooxygenase-1–dependent pathways and respond to ADP despite aspirin and clopidogrel treatment. We did not, however, observe differences in the mean platelet volume, which may reflect platelet age, between the different groups in our study. Second is poor compliance. This is unlikely, however, because the clopidogrel loading dose as well as an aspirin dose were administered in the catheterization laboratory under direct supervision. The second blood sample was drawn 20 to 24 h after this treatment, and there were no differences in AA-induced platelet aggregation between the baseline and post-treatment samples.

Clinical importance. Our study extends previous findings of an association between adverse clinical events and resistance to aspirin (2,5,9) or clopidogrel (23). We evaluated the incidence of CK-MB elevation following PCI, which has been consistently shown to be associated with higher risk of death, MI, and repeat revascularization (24). In accordance with the report by Chen et al. (9), we have found that aspirin-resistant patients had a more than two-fold increase in the incidence of myonecrosis following PCI. Clopidogrel-resistant patients also tended to have more frequent CK-MB elevation compared with clopidogrel-sensitive patients, confirming recent clinical reports (23). Dual drug-resistant patients also had a more than two-fold increase in the rate of myonecrosis compared with dual drug-sensitive patients. This finding supports the recent case-control observation by Wenaweser et al. (25) that among 23 patients with previous stent thrombosis, about half were resistant to the effects of both aspirin and clopidogrel. Taken together, these findings should raise a note of caution that a modest proportion of patients undergoing high-risk PCI may not have adequate antithrombotic protection despite dual antiplatelet therapy.

Study limitations. Our study has several limitations. First, it was powered to evaluate differences in the response to clopidogrel among aspirin-resistant versus aspirin-sensitive patients. However, the sample size was inadequate to estimate the risk of myonecrosis associated with dual drug resistance. Second, the antiplatelet effects of aspirin and clopidogrel were evaluated at two points during a single 24-h period and may not reflect possible temporal fluctuations in individual responses. Nevertheless, these measurements reflect the extent of platelet inhibition just before and following PCI, when optimal inhibition is required. Third, the first blood sample was drawn from an arterial access and the second from a venous access. These conditions were, however, identical for both groups tested. Finally, our study was performed with a clopidogrel loading dose of 300 mg. Recent studies have indicated that a loading dose of 600 mg provides a more rapid and pronounced early response and reduces the rate of clopidogrel resistance (16,26,27). However, most clinical efficacy data have been accrued with the 300 mg dose, and this is the only dose that is currently approved by the U.S. Food and Drug Administration (28).

Conclusions. We have identified a unique group of dual drug-resistant patients who do not achieve adequate antiplatelet effects from either aspirin or clopidogrel. The relatively high incidence of CK-MB elevation after PCI in these patients suggests that they may be at high risk for thrombotic complications following coronary intervention. This finding should be confirmed in a larger-scale study. Nevertheless, the lower response to clopidogrel among aspirin-resistant patients is of particular clinical importance, because clopidogrel has been suggested as alternative therapy for aspirin-resistant patients. Our data would imply that this approach may not be sufficient and that other platelet inhibitors acting on additional targets (other than cyclooxygenase-1 and P2Y12) should be developed and investigated.

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REFERENCES

1. Buchanan MR, Brister SJ. Individual variation in the effects of ASA on platelet function: implications for the use of ASA clinically. Can J Cardiol 1995;11:221–7.
2. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002;105:1650–5.
3. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908–13.
4. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. J Am Coll Cardiol 2005;45:246–51.
5. Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. J Am Coll Cardiol 2003;41:961–5.
6. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001;88:230–5.
7. Grotemeyer KH. Effects of acetylsalicylic acid in stroke patients. Evidence of nonresponders in a subpopulation of treated patients. Thromb Res 1991;63:587–93.
8. Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence of clopidogrel nonresponders among patients with stable angina pectoris scheduled for elective coronary stent placement. Thromb Haemost 2003;89:783–7.
9. Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with a high incidence of myonecrosis after nonurgent percutaneous coronary intervention despite clopidogrel pretreatment. J Am Coll Cardiol 2004;43:1122–6.
10. Macchi L, Christiaens L, Brabant S, et al. Resistance to aspirin in vitro is associated with increased platelet sensitivity to adenosine diphosphate. Thromb Res 2002;107:45–9.
11. Kawasaki T, Ozeki Y, Igawa T, Kambayashi J. Increased platelet sensitivity to collagen in individuals resistant to low-dose aspirin. Stroke 2000;31:591–5.
12. Frelinger AL, Linden MD, Furman MI, et al. Aspirin resistance is associated with markers of platelet activation and is not accounted for by noncompliance or by aspirin underdosing (abstr). Circulation 2004;110 Suppl III:216.
13. Lepantalo A, Virtanen KS, Heikkila J, Wartiovaara U, Lassila R. Limited early antiplatelet effect of 300 mg clopidogrel in patients with aspirin therapy undergoing percutaneous coronary interventions. Eur Heart J 2004;25:476–83.
14. Kleiman NS, Klem J, Fernandes LS, et al. Pharmacodynamic profile of the direct thrombin antagonist bivalirudin given in combination with the glycoprotein IIb/IIIa antagonist eptifibatide. Am Heart J 2002; 143:585–93.
15. Michelson AD, Barnard MR, Krueger LA, Frelinger AL 3rd, Furman MI. Evaluation of platelet function by flow cytometry. Methods 2000;21:259–70.
16. Gurbel PA, Bliden KP, Hayes KM, Yoho JA, Herzog WR, Tantry US. The relation of dosing to clopidogrel responsiveness and the incidence of high post-treatment platelet aggregation in patients undergoing coronary stenting. J Am Coll Cardiol 2005;45:1392–6.
17. Cattaneo M. Aspirin and clopidogrel: efficacy, safety, and the issue of drug resistance. Arterioscler Thromb Vasc Biol 2004;24:1980–7.
18. Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005;352:1293–304.
19. Warala C, Golanski J, Pluta J, et al. Reduced sensitivity of platelets from type 2 diabetic patients to acetylsalicylic acid (aspirin)—its relation to metabolic control. Thromb Res 2004;113:101–13.
20. Tamminen M, Lassila R, Westerbacka J, Vehkavaara S, Yki-Jarvinen H. Obesity is associated with impaired platelet-inhibitory effect of acetylsalicylic acid in nondiabetic subjects. Int J Obes Relat Metab Disord 2003;27:907–11.
21. Keating FK, Whitaker DA, Kabbani SS, Ricci MA, Sobel BE, Schneider DJ. Relation of augmented platelet reactivity to the magnitude of distribution of atherosclerosis. Am J Cardiol 2004;94:725–8.
22. Tschoepe D, Driesch E, Schwippert B, Nieuwenhuis HK, Gries FA. Exposure of adhesion molecules on activated platelets in patients with newly diagnosed IDDM is not normalized by near-normoglycemia. Diabetes 1995;44:890–4.
23. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004;109: 3171–5.
24. Calif RM, Abdelmeguid AE, Kuntz RE, et al. Myonecrosis after revascularization procedures. J Am Coll Cardiol 1998;31:241–51.
25. Wenaweser P, Dorffler-Melly J, Imboden K, et al. Stent thrombosis is associated with an impaired response to antiplatelet therapy. J Am Coll Cardiol 2005;45:1748–52.
26. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. Eur Heart J 2004;25:1903–10.
27. Hochholzer W, Trenk D, Frundi D, et al. Time dependence of platelet inhibition after a 600-mg loading dose of clopidogrel in a large, unselected cohort of candidates for percutaneous coronary intervention. Circulation 2005;111:2560–4.
28. Physician's Desk Reference. 59th edition. Montvale, NJ: Thompson PDR, 2005:1052–5.

Exhibit B

Commentary

HeartDrug

Heart Drug 2001;1:35–43

Variable Interindividual Responses to Antiplatelet Therapies – Do They Exist, Can We Measure Them, and Are They Clinically Relevant?

Insights from the GOLD (AU – Assessing Ultegra) Trial

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Key Words

Platelet(s) · Aspirin · Clopidogrel · Ticlopidine · Glycoprotein IIb/IIIa inhibitors

Abstract

Many patients suffer thrombotic events such as myocardial infarction, stroke and peripheral embolism despite therapy with recommended doses of all currently approved antiplatelet agents. Researchers have suggested that a subset of patients may be resistant to the antiplatelet effects of aspirin, and have developed substantial evidence to support this theory. The thienopyridines ticlopidine and clopidogrel and the glycoprotein IIb/IIIa inhibitors also exhibit substantial interpatient variability in the level of platelet inhibition they achieve. There are several biochemical factors that may contribute to the etiology of individual resistance to antiplatelet medications. Some studies suggest that the variability in patient responsiveness to these drugs may have clinical consequences, and data from trials evaluating clinical end points are needed to further elucidate this correlation.

Introduction

As the role of the platelet in coronary thrombosis becomes clearer, the importance of antiplatelet strategies in acute coronary syndromes (ACS) and percutaneous coronary interventions (PCI) is gaining considerable attention. Aspirin, the first drug found to impede clot formation through its action on platelets, was discovered to profoundly improve the outcome of patients suffering an acute myocardial infarction when the ISIS-2 investigators demonstrated a 21% reduction in mortality among patients on aspirin as compared with those on placebo [1]. It has been subsequently proven that aspirin reduces the incidence of stroke, myocardial infarction and vascular death by 25% in patients with significant risk factors for vascular events [2]. The adenosine diphosphate (ADP)-blocking agents ticlopidine and clopidogrel have been shown to decrease thrombotic events in similar populations of patients to a slightly greater degree [2–5]. Recently, glycoprotein (GP) IIb/IIIa-inhibiting drugs have gained considerable attention for their use in improving outcomes with ACS and PCI and decreasing major adverse cardiac events, including 1-year mortality, following percutaneous revascularization [6–8].

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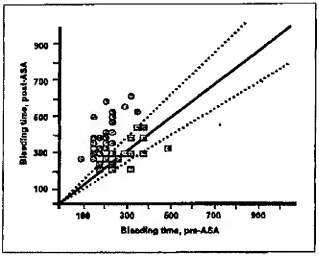

Fig. 1. Variability in bleeding time among aspirin responders (58%) and nonresponders (42%). ASA = Aspirin. The mean variation in bleeding time for responders was 58 ± 10%, and for nonresponders it was 2 ± 4%. Adapted from Buchanan and Brister [12].

An undesirable level of morbidity and mortality following acute coronary events persists despite our increasingly sophisticated arsenal of drugs that hinder the activation and aggregation of platelets in the face of a highly thrombotic environment. A subset of patients treated with the recommended doses of aspirin, a thienopyridine or a GP IIb/IIIa inhibitor may persist in forming new clots, thereby jeopardizing the myocardium and risking arrhythmic and mechanical complications. This brings up certain questions: Do platelet-blocking agents exhibit a degree of unpredictability in therapeutic effect? Do individuals respond differently to antiplatelet medication? Can interpatient variability be measured using platelet function assays, and does this translate into a similar variation in clinical effect and outcome? Can laboratory-guided prescribing of antiplatelet medication improve care by targeting individuals who require elevated doses of drugs or alternative treatments to achieve adequate platelet inactivation? We will attempt to answer these questions with a review of the literature and present an introduction to the GOLD study, the first trial designed to correlate measured platelet function and clinical outcomes in a cohort of patients treated with GP IIb/IIIa inhibitors.

Aspirin

Aspirin irreversibly inhibits platelet cyclooxygenase-1 (COX-1), thereby rendering platelets incapable of synthesizing thromboxane A_2 (TxA_2) for the life of the cell. TxA_2 is released by activated platelets and leads to the recruitment and eventual aggregation of additional newly activated platelets into a nascent thrombus. While aspirin exhibits other effects on the vascular milieu – such as inhibiting prostaglandin [9] and blocking the activity of nitric oxide inhibitors [10] – the effect on TxA_2 is posited to account for the principle antithrombotic effects of the drug.

Alexander et al. [11], in 1999, reported that 63.8% of patients presenting with a non-ST segment elevation ACS were actively taking aspirin. Why this subset of patients experiences a thrombotic event despite chronic antiplatelet therapy is unclear. A possible explanation arises from research that has identified a subset of the population that fails to exhibit the expected platelet inactivation with aspirin. To date, limited data implicate an association between these laboratory measurements of platelet activity and clinical outcomes, suggesting that individuals who receive inadequate platelet inhibition are at greater risk of thrombotic complications than persons who have laboratory evidence of sufficient platelet inhibition.

The concept of aspirin resistance has gained support as researchers have demonstrated significant interpatient variability in measured markers of platelet function in persons taking standard doses of the drug. Clinically, aspirin resistance is defined as the failure of aspirin to prevent thrombotic events. In an attempt to refine the boundaries of aspirin resistance, researchers have used laboratory surrogates – in the form of various platelet function assays – to estimate the prevalence of this entity at as high as 40% (fig. 1) [12].

Several problems exist with respect to such estimations.

First, there is no consensus of opinion as to the most reliable or clinically relevant marker of aspirin-induced platelet blockade. The methods employed most commonly include bleeding time [13], platelet aggregation in response to platelet activators [13–16], platelet aggregation ratio [17, 18] and flow cytometry to detect membrane GPs expressed on activated platelets [15, 19]. The absence of a single 'gold standard' makes comparisons between different studies difficult. Furthermore, only aggregation testing has been significantly linked to clinical outcome data [16, 18].

Second, in vitro aspirin responsiveness appears to differ to some degree based on the dosing used. For example,

in one study of patients undergoing coronary artery bypass surgery after 6 months of therapy with 325 mg of aspirin, 17 of 40 (42%) patients were found to have a bleeding time that failed to prolong more than 2 standard deviations and were deemed aspirin nonresponders [12]. In another arm of the same study, only 3 out of 10 healthy volunteers showed prolongation in bleeding time at a dose of 80 mg daily. When the aspirin dose was increased to 1,300 mg daily, 6 of the 7 remaining volunteers had prolonged bleeding times. The authors concluded that most volunteers who were aspirin nonresponders at the low doses of aspirin would respond to the higher dose of aspirin and suggested that the 42% aspirin resistance found in the cohort of coronary artery bypass graft patients might change with increased dosing of aspirin. The concept of a dose-dependant in vitro response to aspirin was documented by a trial that measured platelet aggregation in 107 patients on varying doses of aspirin for stroke prevention [14]. At an aspirin dose of 325 mg daily, inhibition of platelet aggregation was complete in 79% of patients, with 21% of the subjects exhibiting only partial blockade. Escalating the dosage to 1,300 mg in 12 persons in the latter group resulted in complete inhibition in all but 3 patients.

Clinically, however, a dose response has not been found. Dosing aspirin at 100 mg proved to be no less effective than 1,000 mg in preventing restenosis after femoropopliteal percutaneous transluminal angioplasty [20]. A meta-analysis reviewing aspirin dosing in 11 randomized, placebo-controlled trials concluded that all doses from 50 to 1,500 mg daily produced the same reduction in stroke risk (15%) in patients with a history of cerebrovascular disease [21]. In fact, some data are more suggestive of a greater clinical benefit with lower doses of aspirin. A meta-analysis of low- versus high-dose aspirin suggested a better outcome with smaller doses of the drug [22]. A recent randomized trial of aspirin dosing in patients undergoing carotid endarterectomy concluded that patients taking 325 mg or less of aspirin had fewer adverse events within 3 months than did the patients taking 650–1,300 mg of aspirin daily [23].

Third, in addition to significant interpatient variability, there may exist some degree of intrapatient variability. Researchers who measured platelet aggregation in 171 stroke patients found that 154 subjects attained complete platelet inhibition on doses of aspirin varying from 325 to 1,300 mg per day [24]. Of these 154 subjects that presumably had an adequate response to aspirin, 47 (30.5%) did not maintain that effect upon repeated testing, despite fulfilling the criteria of regular compliance checks. The results of this trial suggest that aspirin responsiveness is dynamic over time in many individuals. Such a notion was challenged by a small trial that evaluated platelet aggregation in 31 healthy, young adults and demonstrated that once platelet inhibition was achieved, it was maintained throughout the duration of the 28 days of prolonged aspirin ingestion [25]. In another small series of healthy men taking 324 mg of aspirin daily, bleeding time and platelet aggregation were found to be constant in each individual on separate assessments 30 months apart [26].

Mechanisms of Aspirin Realstance

The mechanism underlying aspirin resistance has yet to be fully elucidated, but a number of factors have been shown to cause decreased aspirin efficacy alone or in combination with other variables. Aspirin inhibits COX-1 from metabolizing arachidonic acid to the potent platelet agonist TxA_2, but the drug has little to no effect on lipoxygenase [12]. Lipoxygenase converts arachidonic acid into 12-hydroxyeicosatetraenoic acid (12-HETE), a metabolite that increases platelet adhesivity. When platelet COX-1 is inhibited, platelet 12-HETE synthesis via the lipoxygenase pathway may increase. It is conceivable that lipoxygenase could be more prevalent or more active in persons who show resistance to aspirin. In a study by Buchanan and Brister [12], platelet 12-HETE synthesis and platelet adhesivity remained unchanged or became enhanced with aspirin therapy in the patients classified as aspirin nonresponders, whereas aspirin responders all showed decreased 12-HETE production with aspirin therapy.

Aspirin completely blocks COX-1, but its effect on COX-2 is 170 times weaker [27]. In aspirin-resistant patients, the persistent production of TxA_2 may occur as the result of unusually enhanced COX-2 activity. Furthermore, COX-2 synthesis, even in normal patients, can be rapidly induced by proinflammatory or mitogenic stimuli, including cytokines, endotoxin and growth factors [28]. Since TxA_2 needs to be blocked by 95–99% to inhibit platelet aggregation [29], even small amounts of COX-2-produced TxA_2 could feasibly result in clinically significant aspirin failure. It has been suggested that high platelet turnover can enhance platelet COX-2 expression and thereby inhibit the effect of aspirin on platelet aggregation [30]. One study found that 20% of patients with unstable angina treated with aspirin had unusually high rates of thromboxane metabolite in 6- to 8-hour urine collections, suggesting persistent thromboxane production despite aspirin therapy [31].

Aspirin is able to irreversibly acetylate the COX-1 that is present not only in platelets, but also in monocytes/macrophages and vascular endothelial cells. While this results in inhibition for the life span of the affected platelet, nucleated cells are capable of synthesizing new COX-1. The plasma half-life of aspirin is too brief (15–20 min) to suppress extra-platelet COX-1 throughout the drug-dosing interval. Indobufen, a reversible inhibitor of COX-1 with a half-life of 8 h, proved to suppress the rate of TxA_2 biosynthesis better than aspirin when given to patients with unstable angina [32]. This supports the speculation that nucleated cells in the vessel serve as a reservoir of TxA_2 synthesis.

The platelet GP complex IIb/IIIa, which acts as a receptor for fibrinogen and other adhesive molecules, is required for platelet aggregation. A polymorphism in the gene encoding GP IIIa results in the presence of two alleles in the population: PlA^1 and PlA^2. The presence of the allele PlA^2 has been suggested as a heritable risk factor for coronary artery disease [33]. In an evaluation of patients suffering myocardial infarction, researchers found a 50% incidence of the A2 allele compared with 27% in age- and sex-matched control subjects [34]. It appears that this genetic variable may also play a role in an individual's response to aspirin. One study found that aspirin therapy might be associated with elevated thrombin levels in persons with the PlA^2 allele [35]. However, this issue is far from settled. Two other studies produced results that counter the suggestion that the PlA^2 allele is culpable for resistance to aspirin therapy, by showing enhanced inhibition of platelet aggregation with aspirin in individuals who are heterozygous for the PlA^2 allele [36, 37].

Kawasaki et al. [26] suggest that aspirin resistance is a function of the sensitivity of platelets to collagen. They demonstrated that the collagen concentration required to trigger platelet aggregation in aspirin nonresponders is half the concentration needed to stimulate platelet aggregation in aspirin responders. This difference was seen both with and without aspirin, and on two separate measurements performed 30 months apart.

Other mechanisms that may play a role in thrombosis despite aspirin therapy are variable rates of aspirin hydrolysis [38, 39], platelet stimulation via shear stress, ADP and endothelial prostacyclin [28] and variability in platelet aggregation related to a polymorphism of the platelet arginine vasopressin V_1 receptor [40]. Tobacco use [41], hyperlipidemia [42], testosterone level [43] and exercise [44] also affect platelet inhibition in response to aspirin.

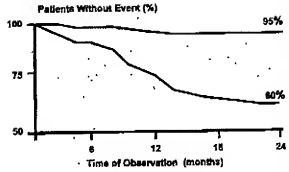

Fig. 2. Rate of major cardiac events among aspirin responders (n = 114) and nonresponders (n = 60) ($p < 0.0001$). Adapted from Grotemeyer et al. [18].

Clinical Relevance of Aspirin Resistance

While there are many studies demonstrating variable levels of platelet function with aspirin therapy, few trials have been performed showing that this concept translates into clinical significance. Grotemeyer et al. [18] evaluated 176 stroke victims upon their discharge from hospital and classified them as aspirin responders or nonresponders based on platelet function 12 h after a 500-mg oral dose of aspirin. All patients were treated with 1,500 mg of aspirin per day and followed for 24 months for the major end points of stroke, myocardial infarction or vascular death. Major end points were seen in only 5 of the 114 (4.4%) aspirin responders, but in 24 out of 60 (40%) nonresponders ($p < 0.0001$), suggesting more thromboembolic events in patients with poor platelet inhibition (fig. 2).

Another study evaluated 70 male and 30 female patients with intermittent lower extremity claudication who were undergoing percutaneous balloon angioplasty at the level of the iliaco-femoral artery [16]. The researchers measured the platelet reactivity in response to ADP and collagen after the patients were placed on aspirin at a dose of 100 mg per day. During the subsequent 18 months of clinical observation, eight patients suffered reocclusion at the site of the angioplasty. Comparisons of the aggregation results in this group revealed that restenosis occurred exclusively in male patients who failed to achieve inhibition of platelet aggregation in response to both ADP and collagen. The relative risk for reocclusion in the patients who did not respond appropriately to aspirin was 1.871 ($p = 0.00093$).

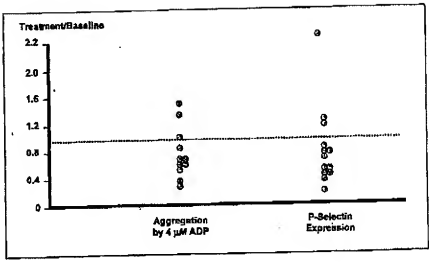

Fig. 3. Variability of platelet aggregation and P-selectin expression among 9 healthy volunteers treated with 250 mg of ticlopidine twice a day and 325 mg of aspirin daily for 5 days. Note the wide variation in treatment-to-baseline ratio among in vitro measurements. Adapted from Farrell et al. [15].

An ongoing trial assessing long-term cardiovascular events in patients with coronary disease has shown that 8–12% of patients taking aspirin do not achieve the therapeutic benefit of platelet inhibition, based on aggregometry [45]. The results of this study should shed light on the clinical relevance of laboratory-measured aspirin resistance.

As shown, platelet function studies reveal a significant variation in an individual's response to aspirin and suggest that a subset of the population might be resistant to the drug's protective effects against thromboembolic complications. What is less clear is whether the mechanisms that confer aspirin resistance also affect an individual's response to other antiplatelet medications.

Ticlopidine and Clopidogrel

The thienopyridines, ticlopidine (Ticlid) and clopidogrel (Plavix), irreversibly inhibit platelet aggregation by preventing ADP-mediated structural alterations in the GP IIb/IIIa receptor, thereby inhibiting platelet binding to fibrinogen [46]. Both drugs, when used chronically in the place of aspirin, have been shown to be slightly more effective than aspirin in the secondary prevention of thrombotic events [3, 5]. When dosed simultaneously, the thienopyridines and aspirin have synergistic antiplatelet effects [47].

Although not as well studied as with aspirin, interindividual variability has also been observed in platelet reactivity during treatment with this class of medication. Farrell et al. [15] studied platelet aggregation in healthy subjects treated with 250 mg of ticlopidine twice daily for 5 days. Substantial variation in aggregation response to ADP was seen among the patients in this cohort; 15% of specimens revealed increased aggregation with ticlopidine. Flow cytometric determination of P-selectin, a surface protein expressed on activated platelets, similarly showed a range of drug effect that varied from full platelet inhibition to little or none. Blood samples from a group of patients taking 75 mg of clopidogrel daily demonstrated a similar range of effect [48]. ADP-induced aggregation in healthy subjects on 75 mg daily showed a mean level of platelet inhibition that was within the therapeutic range on day 2 of treatment, but the variability in the group was ±27% from the mean (fig. 3).

Variable levels of platelet inhibition have therefore been documented among patients in these small series. Does this imply that a subset of the population will be thienopyridine nonresponders and, as seen with aspirin, exhibit ticlopidine or clopidogrel resistance? Unfortunately, no studies have been performed to assess whether patients with decreased in vitro effect from therapy with thienopyridines suffer more thrombotic complications.

GP IIb/IIIa Inhibitors

The platelet GP IIb/IIIa receptor is a platelet-specific integrin that mediates platelet aggregation, binding fibrinogen and von Willebrand factor in a common response of platelets to stimulation by all agonists. The inhibitors of

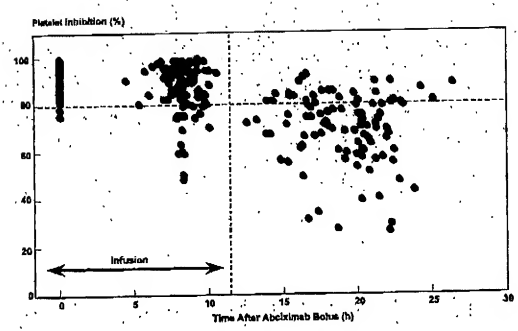

Fig. 4. Variability in platelet inhibition (as measured by aggregometry) following bolus and infusion of abciximab. At 12–20 h there is wide variation in platelet function, ranging from >80% to <30%. Adapted from Steinhubl et al. [50].

GP IIb/IIIa represent a class of drugs that compete with fibrinogen for occupancy of its platelet receptor and thereby restrict platelet aggregation. Abciximab (ReoPro), a monoclonal antibody Fab fragment, and other naturally occurring and synthetic peptide and nonpeptide antagonists of the GP IIb/IIIa receptor have been thoroughly studied and proven to limit thrombotic complications during acute coronary events and after coronary interventions.

The initial animal studies with abciximab suggested that blockade of >80% of the platelet GP IIb/IIIa receptors – with a corresponding ≥ 80% inhibition of platelet aggregation – is necessary to arrest thrombosis in a thrombogenic environment [49]. In the largest study to date, platelet aggregation was measured in 97 patients receiving abciximab in conjunction with coronary angioplasty [50]. The degree of platelet inhibition was evaluated immediately after abciximab bolus (0.25 mg/kg), 8 h after beginning the 12-hour infusion (0.125 μg/kg/min) and the following day (13–26 h after the bolus). All patients but one achieved >80% platelet inhibition immediately after the infusion. Eight hours after the bolus, but still within the infusion period, 13% of the patients had a level of platelet blockade under 80%, implying a loss of meaningful protection against thrombosis in this group. By the next morning (13–26 h after the bolus), only 29% of patients continued to have >80% inhibition of platelet activity (fig. 4). This study demonstrated a substantial variability in the capacity of abciximab to impede platelet aggregation in patients undergoing coronary angioplasty that was not predictable based on any clinical or hematological parameters, and uncovered a subset of patients that may be refractory to the antithrombotic effects of the recommended doses of abciximab. Interestingly, although this study was not designed to evaluate clinical outcomes, the investigators did find a significant increase in risk for adverse events in those patients with less than 80% platelet inhibition at 8 h.

Other studies in limited numbers of patients have similarly reflected a substantial heterogeneity in individual response to therapy with abciximab and other GP IIb/IIIa inhibitors [51–56]. One study suggested that patients with unstable angina are more refractory to the antiplatelet effects of GP IIb/IIIa blockade than patients with stable

angina [57]. The clinical impact of this heterogeneity in a large population receiving GP IIb/IIIa inhibitors is currently unknown.

The GOLD Study

The purpose of the GOLD (from the chemical symbol for gold: AU – Assessing Ultegra) study is to identify the level of platelet inhibition at several time points in patients undergoing a PCI who are being treated with a GP IIb/IIIa inhibitor, and to establish what level of inhibition is associated with the fewest thrombotic complications. The study will also determine what percentage of patients achieves this level [58]. It will prospectively evaluate the degree of platelet inhibition in 500 patients undergoing PCI who are being treated with any of the three currently approved GP IIb/IIIa inhibitors – abciximab, eptifibatide and tirofiban. In vitro platelet evaluation will be performed using the Ultegra-Rapid Platelet Function Assay (RPFA) (Accumetrics, San Diego, Calif., USA), an automated device that assesses platelet function in whole blood utilizing the ability of activated platelets to bind fibrinogen. Fibrinogen-coated polystyrene microparticles agglutinate in whole blood in proportion to the number of unblocked platelet GP IIb/IIIa receptors. Pharmacological blockade of GP IIb/IIIa receptors prevents this interaction and subsequently diminishes agglutination in proportion to the degree of receptor blockade achieved [59]. The Ultegra-RPFA has been validated against aggregometry in 120 patients undergoing PCI and treated with a GP IIb/IIIa inhibitor [60]. Enrollment in the GOLD study concluded in late 1999. Preliminary results were presented at the 2000 American College of Cardiology Meeting and demonstrated a significant correlation between the level of platelet inhibition and the occurrence of major adverse cardiac events.

Summary

The concept of variable patient response to medication is not new to clinicians. It is common to expect patients with diabetes, hyperlipidemia and hypertension to demonstrate individual nuances in their response to medications used to treat these disorders. The physician monitors the efficacy of treatment using various physical examination findings and laboratory values and makes adjustments as needed to optimize care.

Such is not the case with antiplatelet therapy. The current practice is to place all patients on the standard doses of antiplatelet agents and assume adequate protection from thrombotic complications without monitoring platelet function in each individual. With new evidence suggesting significant heterogeneity in individual responses to all antiplatelet medications, a move toward laboratory-guided platelet inhibition may be warranted.

Still, the question remains: if we identify a patient whose platelet function is not amply inhibited, what adjustment in therapy should we make to minimize thrombogenesis? Some research [12, 14, 24] suggests that aspirin resistance is at least partly a dose-dependant phenomenon and that dose escalation in targeted individuals might enhance efficacy. But even these studies found a minority of patients who failed to exhibit appropriate platelet inactivation at the maximum aspirin dose. At least one small trial has found that patients whose platelet function was insufficiently inhibited on 81 mg of aspirin demonstrated a paradoxical increase in platelet activation on higher doses [61]. Importantly, clinical trials have not uniformly supported the rationale of using higher dosing of antiplatelet medication to overcome the effect of drug resistance [20–23]. These data suggest that alternative methods of platelet blockade must be sought for patients resistant to all doses of conventional antiplatelet medication. At this point, the treatment strategy for such patients is far from perspicuous and may ultimately entail a combination of dosing changes and alternative medications.

Variable degrees of platelet blockade, based on in vitro assays of platelet function, have been consistently demonstrated in persons taking aspirin, thienopyridines and GP IIb/IIIa inhibitors. Small studies have suggested the clinical importance of this resistance to therapy in aspirin-treated patients. It appears that certain patients treated with GP IIb/IIIa inhibitors are in jeopardy of increased thrombotic complications if they fail to show an adequate response to the drug as measured in the clinical laboratory.

Despite the critical role of antiplatelet therapies in the treatment of cardiovascular diseases, there remain substantial gaps in our knowledge regarding the clinical impact of inadequate platelet blockade in persons receiving aspirin, ticlopidine, clopidogrel or the GP IIb/IIIa inhibitors. The GOLD study is the first large-scale clinical trial to identify a link between measured platelet function and clinical outcomes in patients receiving GP IIb/IIIa-inhibiting drugs in the setting of coronary intervention. Its results are the first step towards prospective treatment trials guided by individual monitoring of patient platelet response to this class of medications.

References

1 Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Lancet 1988;ii:349–360.

2 Collaborative overview of randomised trials of antiplatelet therapy – I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet Trialists' Collaboration. BMJ 1994;308:81–106.

3 Hass WK, Easton JD, Adams HP Jr, Pryse-Phillips W, Molony BA, Anderson S, Kamm B: A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. Ticlopidine Aspirin Stroke Study Group. N Engl J Med 1989; 321:501–507.

4 Gent M, Blakely JA, Easton JD, Ellis DJ, Hachinski VC, Harbison JW, Panak E, Roberts RS, Sicurella J, Turpie AGG: The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. Lancet 1989;i:1216–1220.

5 A randomised, blinded trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. Lancet 1996;348:1329–1339.

6 Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. The EPIC Investigation. N Engl J Med 1994;330:956–961.

7 Inhibition of the platelet glycoprotein IIb/IIIa receptor with tirofiban in unstable angina and non-Q-wave myocardial infarction. Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) Study Investigators. N Engl J Med 1998;338:1488–1497.

8 Randomised placebo-controlled and balloon-angioplasty-controlled trial to assess safety of coronary stenting with use of platelet glycoprotein-IIb/IIIa blockade. The EPISTENT Investigators. Evaluation of Platelet IIb/IIIa Inhibitor for Stenting. Lancet 1998;352:87–92.

9 Komatsu H, Yaju H, Chiba K, Okamoto T: Inhibition by cyclo-oxygenase inhibitors of interleukin-6 production by human peripheral blood mononuclear cells. Int J Immunopharmacol 1991;13:137–1146.

10 Rosenblum W, Nishimura H, Nelson G: L-NMMA in brain microcirculation of mice is inhibited by blockade of cyclooxygenase and by superoxide dismutase. Am J Physiol 1992;262: H1343–H1349.

11 Alexander JH, Harrington RA, Tuttle RH, Berdan LG, Lincoff AM, Deckers JW, Simoons ML, Guarei A, Hochman JS, Wilcox RG, Kitt MM, Eisenberg PR, Califf RM, Topol EJ, Karsh K, Ruzyllo W, Stepinska J, Widimsky P, Boland JB, Armstrong PW: Prior aspirin use predicts worse outcomes in patients with non-ST-elevation acute coronary syndromes. PURSUIT Investigators. Platelet IIb/IIIa in Unstable angina: Receptor Suppression Using Integrilin Therapy. Am J Cardiol 1999;83:1147–1151.

12 Buchanan MR, Brister SJ: Individual variation in the effects of ASA on platelet function: Implications for the use of ASA clinically. Can J Cardiol 1995;1:221–227.

13 Born G: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962;194:927–929.

14 Helgason C, Tortorice K, Winkler S, Penney D, Schuler J, McClelland T, Brace L: Aspirin response and failure in cerebral infarction. Stroke 1993;24:345–350.

15 Ferrell T, Hayes K, Sobel B, Schneider D: The lack of augmentation by aspirin of inhibition of platelet reactivity by ticlopidine. Am J Cardiol 1999;83:770–774.

16 Mueller MR, Salat A, Stangl P, Murabito M, Palaki S, Boehm D, Koppensteiner R, Ergun E, Mittlboeck M, Schreiner W, Lassel U, Wolner E: Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. Thromb Haemost 1997;78:1003–1007.

17 Wu K, Hoak J: A new method for the quantitative detection of platelet aggregates in patients with arterial insufficiency. Lancet 1974;ii:924–926.

18 Grotemeyer K-H, Scharfinski H-W, Husstedt J-W: Two-year follow-up of aspirin responder and aspirin non-responder. A pilot study including 180 post-stroke patients. Thromb Res 1993;71:397–403.

19 Valtetsa N, Morgan CD, Reis M: Aspirin resistance using flow cytometry (abstract). Blood 1997;10:124b.

20 Minar E, Ahmadi A, Koppensteiner R, Maca T, Stumpflen A, Ugurlucan A, Ehringer H: Comparison of effects of high-dose and low-dose aspirin on restenosis after femoropoplitial percutaneous transluminal angioplasty. Circulation 1995;91:2167–2173.

21 Johnson E, Lanes S, Wentworth CI, Satterfield M, Abeba B, Dicker L: A metaregression analysis of the dose-response effect of aspirin on stroke. Arch Intern Med 1999;159:1248–1253.

22 Verheugt F: Differential dose effect of aspirin in the primary prevention of myocardial infarction. J Am Coll Cardiol 1998;31(suppl A): 352A.

23 Taylor DW, Barnett HJM, Haynes RB, Ferguson GG, Sackett DL, Thorpe KE, Simard D, Silver FL, Hachinski V, Clagett GP, Barnes R, Spence JD: Low-dose and high-dose acetylsalicylic acid for patients undergoing carotid endarterectomy: A randomised controlled trial. ASA and Carotid Endarterectomy (ACE) Trial Collaborators. Lancet 1999;353:2179–2184.

24 Helgason CM, Bolin KM, Hoff JA, Winkler SR, Mangat A, Tortorice KL, Brace LD: Development of aspirin resistance in persons with previous ischemic stroke. Stroke 1994;25: 2331–2336.

25 Pappas JM, Wessengard JC, Bull BS: Population variability in the effect of aspirin on platelet function. Implications for clinical trials and therapy. Arch Pathol Lab Med 1994;118:801–804.

26 Kawasaki T, Ozeki Y, Igawa T, Kambayashi J: Increased platelet sensitivity to collagen in individuals resistant to low-dose aspirin. Stroke 2000;31:591–595.

27 Weber A-A, Zimmermann KC, Meyer-Kirchrath J, Schror K: Cyclooxygenase-2 in human platelets as a possible factor in aspirin resistance. Lancet 1999;353:900.

28 Schror K: Aspirin and platelets: The antiplatelet action of aspirin and its role in thrombosis treatment and prophylaxis. Semin Thromb Hemost 1997;23:349–356.

29 Reilly IAG, FitzGerald GA: Inhibition of thromboxane formation in vivo and ex vivo: Implications for therapy with platelet inhibitory drugs. Blood 1987;69:180–186.

30 Zimmerman N, Klenzle P, Weber A, Gams E, Hohlfeld T, Schror K: Platelet aspirin resistance is associated with an increased platelet content of cyclooxygenase-2. Circulation 1999; 100(suppl):I-327.

31 Vejar M, Fragnaso G, Hackett D, Lipkin P, Maseri A, Bora G, Ciabattoni G, Patrono C: Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina. Thromb Haemost 1990;63:163–168.

32 Cipollone F, Patrignani P, Greco A, Panara MR, Padovano R, Cuccurullo F, Patrono C, Rebuzzi AG, Liuzzo G, Quaranta G, Maseri A: Differential suppression of thromboxane biosynthesis by ibuprofen and aspirin in patients with unstable angina. Circulation 1997;96: 1109–1116.

33 Weiss EJ, Bray PF, Tayback M, Schulman SP, Kickler TS, Becker LC, Weiss JL, Gerstenblith G, Goldschmidt-Clermont PJ: A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 1996;334:1090–1094.

34 Carter A, Ossei-Gerning N, Wilson I, Grant P: Association of the platelet PlA(A) polymorphism of glycoprotein IIb/IIIa and the fibrinogen B beta 448 polymorphism with myocardial infarction and extent of coronary artery disease. Circulation 1997;96:1424–1431.

35 Undas A, Sanak M, Musial J, Szczeklik A: Platelet glycoprotein IIIa polymorphism, aspirin, and thrombin generation. Lancet 1999; 353:982–983.

36 Cooke GE, Bray PF, Hamlington JD, Pham DM, Goldschmidt-Clermont PJ: PIA2 polymorphism and efficacy of aspirin. Lancet 1998; 351:1253.

37 Michelson A, Furmen M, Goldschmidt-Clermont P, Mascelli M, Hendolin C, Coleman L, Hamlington J, Barnard M, Kickler T, Christie D, Kundu S, Bray P: Platelet GP IIIa PIA polymorphisms display different sensitivities to agonists. Circulation 2000;101:1013–1018.

38 Ryan L, Hakeakamp K: Variable response to aspirin measured by platelet aggregation and bleeding time. Lab Med 1991;22:197–202.

39 Akopov S, Grigorian G, Gabrielian E: Dose-dependent aspirin hydrolysis and platelet aggregation in patients with atherosclerosis. J Clin Pharmacol 1992;32:133–135.

40 Lachant N, Smith M, Xie Z, Romani W: Heterogeneity of the aggregation response of human platelets to arginine vasopressin. Am J Hematol 1995;49:56–66.
41 Davis JW, Hartman GR, Lewis HD, Shelton L, Eigenberg DA, Hassanein KM, Higuite CE, Rottinger HA: Cigarette smoking-induced enhancement of platelet function: Lack of prevention by aspirin in men with coronary artery disease. J Lab Clin Med 1985;105:479–483.
42 Szczeklik A, Musial J, Undas A: Inhibition of thrombin generation by aspirin is blunted in hypercholesterolemia. Arterioscler Thromb Vasc Biol 1996;16:948–954.
43 Sprangar M, Aspey BS, Harrison MJG: Sex difference in antithrombotic effect of aspirin. Stroke 1989;20:34–37.
44 Li N, Wallen H, Hjemdahl P: Evidence for prothrombotic effects of exercise and limited protection by aspirin. Circulation 1999;100:1374–1379.
45 Poggio ED, Kottke-Marchant K, Walsh PA, Brooks LM, Dela Rosa LR, Topol EJ: The prevalence of aspirin resistance in cardiac patients as measured by platelet aggregation and PFA-100 (abstract). J Am Coll Cardiol 1999; 33:254A.
46 Mills DCB, Puri R, Hu C-J, Minniti C, Grana G, Freedman MD, Colman RF, Colman RW: Clopidogrel inhibits the binding of ADP analogues to the receptor mediating inhibition of platelet adenylate cyclase. Arterioscler Thromb 1992;12:430–436.
47 Rupprecht H-J, Darius H, Borkowski U, Voigtlander T, Nowak B, Genth S, Meyer J: Comparison of antiplatelet effects of aspirin, ticlopidine, or their combination after stent implantation. Circulation 1998;97:1046–1052.
48 Thebault J, Kieffer G, Lowe G, Nimmo W, Carlou R: Repeated-dose pharmacodynamics of clopidogrel in healthy subjects. Semin Thromb Hemost 1999;25:9–14.
49 Coller BS, Scudder LE, Beer J, Gold HK, Folts JD, Cavagnaro J, Jordan R, Wagner C, Iullucci J, Knight D, Ghrayeb J, Smith C, Weisman HF, Berger H: Monoclonal antibodies to platelet glycoprotein IIb/IIIa as antithrombotic agents. Ann NY Acad Sci 1991;614:193–213.
50 Steinhubl SR, Kottke-Marchant K, Moliterno DJ, Rosenthal ML, Godfrey NK, Coller BS, Topol EJ, Lincoff AM: Attainment and maintenance of platelet inhibition by standard dosing of abciximab in diabetic and nondiabetic patients undergoing percutaneous coronary intervention. Circulation 1999;100:1977–1982.
51 Kereiakes DJ, Broderick TM, Roth EM, Whang D, Shimshak T, Runyon JP, Haltemer C, Scheinder J, Lacock P, Mueller M, Abbottamin CW: Time course, magnitude, and consistency of platelet inhibition by abciximab, tirofiban, or eptifibatide in patients with unstable angina pectoris undergoing percutaneous coronary intervention. Am J Cardiol 1999;84: 391–395.
52 Kleiman NS, Raizner AE, Jordan R, Wang AL, Norton D, Mace KF, Joshi A, Coller BS, Weisman HF: Differential inhibition of platelet aggregation induced by adenosine diphosphate or a thrombin receptor-activating peptide in patients treated with bolus chimeric 7E3 Fab: Implications for inhibition of the internal pool of GPIIb/IIIa receptors. J Am Coll Cardiol 1995;26:1665–1671.
53 Harrington RA, Kleiman NS, Kottke-Marchant K, Lincoff AM, Tcheng JE, Signoo KN, Joseph D, Rios G, Trainor K, Rose D, Greenberg CS, Kitt MM, Topol EJ, Califf RM: Immediate and reversible platelet inhibition after intravenous administration of a peptide glycoprotein IIb/IIIa inhibitor during percutaneous coronary intervention. Am J Cardiol 1995;76:1222–1227.
54 Mascelli MA, Worley S, Veriabo NJ, Lance ET, Mack S, Schaible T, Weisman HF, Jordan RE: Rapid assessment of platelet function with a modified whole-blood aggregometer in percutaneous transluminal coronary angioplasty patients receiving anti-GPIIb/IIIa therapy. Circulation 1997;96:3860–3866.
55 Mascelli MA, Lance ET, Damaraju L, Wagner CL, Weisman HF, Jordan RE: Pharmacodynamic profile of short-term abciximab treatment demonstrates prolonged platelet inhibition with gradual recovery from GP IIb/IIIa receptor blockade. Circulation 1998;97:1680–1688.
56 Simoons ML, de Boer MJ, van den Brand MJBM, van Miltenburg AJM, Hoorntje JCA, Heyndrickx GR, van der Wieken LR, De Bono D, Rutsch W, Schaible TF, Weisman HF, Klootwijk P, Nijsen KM, Sibbe J, de Feyter PJ: Randomized trial of a GPIIb/IIIa platelet receptor blocker in refractory unstable angina. European Cooperative Study Group. Circulation 1994;89:596–603.
57 Catella-Lawson F, Kapoor S, Moretti DT, Combe S, Nicolas S, Dorey S, Theroux P, FitzGerald GA, AFIRME Investigators: Chronic oral glycoprotein IIb/IIIa antagonism in patients with unstable coronary syndromes: Reduced antiplatelet effect in comparison to patients with stable coronary artery disease. Circulation 1998;98:I-251.
58 Steinhubl S: Assessing the optimal level of platelet inhibition with GPIIb/IIIa inhibitors in patients undergoing coronary intervention. Rationale and design of the GOLD study. J Thromb Thrombolysis 2000;9:199–205.
59 Coller BS, Lang D, Scudder LE: Rapid and simple platelet function assay to assess glycoprotein IIb/IIIa receptor blockade. Circulation 1997;95:860–867.
60 Smith JW, Steinhubl SR, Lincoff AM, Coleman JC, Lee TT, Hillman RS, Coller BS: Rapid platelet-function assay: An automated and quantitative cartridge-based method. Circulation 1999;99:620–625.
61 Farrell TF, Hayes KA, Tracy PB, Sobel BE, Schneider DJ: Unexpected, discordant effects of aspirin on platelet reactivity (abstract). J Am Coll Cardiol 1998;31:352A.

Exhibit C

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Aspirin Resistance Is Associated With a High Incidence of Myonecrosis After Non-Urgent Percutaneous Coronary Intervention Despite Clopidogrel Pretreatment

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OBJECTIVES We sought to investigate the effect of aspirin resistance on the incidence of myonecrosis after non-urgent percutaneous coronary intervention (PCI) among patients pretreated with clopidogrel.

BACKGROUND Oral antiplatelet therapy using aspirin and a thienopyridine is the standard of care for preventing thrombotic complications of PCI. The effect of aspirin resistance on the outcomes of patients undergoing PCI is unknown.

METHODS We used the Ultegra Rapid Platelet Function Assay-ASA (Accumetrics Inc., San Diego, California) to determine aspirin responsiveness of 151 patients scheduled for non-urgent PCI. All patients received a 300-mg loading dose of clopidogrel >12 h before and a 75-mg maintenance dose in the morning of the PCI. The incidence of myonecrosis was measured by creatine kinase-myocardial band (CK-MB) and by troponin I (TnI) elevations after PCI.

RESULTS A total of 29 (19.2%) patients were noted to be aspirin-resistant. There was a significantly higher incidence of female subjects in the aspirin-resistant versus aspirin-sensitive groups. The incidence of any CK-MB elevation was 51.7% in aspirin-resistant patients and 24.6% in aspirin-sensitive patients ($p = 0.006$). Elevation of TnI was observed in 65.5% of aspirin-resistant patients and 38.5% of aspirin-sensitive patients ($p = 0.012$). Multivariate analysis revealed aspirin resistance (odds ratio [OR] 2.9; 95% confidence interval [CI] 1.2 to 6.9; $p = 0.015$) and bifurcation lesion (OR 2.8; 95% CI 1.3 to 6.0; $p = 0.007$) to be independent predictors of CK-MB elevation after PCI.

CONCLUSIONS Despite adequate pretreatment with clopidogrel, patients with aspirin resistance as measured by a point-of-care assay have an increased risk of myonecrosis following non-urgent PCI. (J Am Coll Cardiol 2004;43:1122–6) © 2004 by the American College of Cardiology Foundation

Early complications of percutaneous coronary intervention (PCI) are caused by arterial thrombosis at the site of vessel injury (1). More complete platelet inhibition using aspirin and a thienopyridine during PCI offers protection against ischemic complications (2–7). However, 8% to 45% (8–11) of patients do not respond to aspirin therapy as determined

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by different laboratory tests, and these aspirin-resistant patients are at increased risk of thrombotic events (8,12–14). The contribution of aspirin, a relatively weak antiplatelet agent, to the prevention of thrombotic complications of PCI in the presence of the full effect of a thienopyridine is unknown. This study was designed to compare the incidence of myonecrosis after PCI between aspirin-resistant and aspirin-sensitive patients pretreated with clopidogrel >12 h before PCI.

METHODS

Study population. Consecutive patients with aspirin use of 80- to 325-mg daily for ≥1 week and who were scheduled for PCI were enrolled. Exclusion criteria included saphenous vein graft intervention, chronic total occlusions that could not be crossed by guidewires, preprocedural elevation of creatine kinase-myocardial band (CK-MB) or troponin I (TnI), planned use of glycoprotein IIb/IIIa inhibitors, and the use of antiplatelet drugs other than aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) within two weeks of the PCI. The local ethics committee on human research approved the protocol, and all patients provided written informed consent.

Study protocol. After collecting baseline blood samples for CK-MB, TnI, and aspirin responsiveness, all patients received an oral loading dose of 300-mg clopidogrel 12 to 24 h before the procedure. The PCI procedure was performed on the following day according to standard practice, after the patients received an additional 75-mg maintenance dose of clopidogrel. Unfractionated heparin 70 U/kg or enoxaparin 1 mg/kg was used for procedural anticoagulation at operator discretion. Following the procedure, blood samples for TnI were collected at 12 to 24 h, whereas those for CK-MB were collected at 6 to 8 h. If CK-MB was elevated, serial measurements every 8 h were obtained and the peak level was recorded. The CK-MB was considered elevated if ≥16 U/l, which was further subdivided into 1 to 3 × (16 to 48 U/l), 3 to 5 × (49 to 80 U/l), and >5 × (>80 U/l) normal. A TnI value of ≥2.0 ng/ml was considered elevated.

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Abbreviations and Acronyms

ARU = aspirin reaction unit
CK-MB = creatine kinase-myocardial band
NSAID = non-steroidal anti-inflammatory drugs
OR = odds ratio
PCI = percutaneous coronary intervention
TnI = troponin I

Aspirin-induced platelet inhibition was measured using a commercially available point-of-care assay, the Ultegra Rapid Platelet Function Assay-ASA (RPFA-ASA) (Accumetrics Inc., San Diego, California). Citrate-anticoagulated blood 2 ml was added to RPFA-ASA cartridges, which contain fibrinogen-coated beads and platelet agonists. If aspirin has produced the expected antiplatelet effect, fibrinogen-coated beads will not agglutinate, and light transmission will not increase. The result is expressed as aspirin reaction unit (ARU). An ARU ≥550 indicates the absence of aspirin-induced platelet dysfunction, based on correlation with epinephrine-induced light transmission aggregometry in aspirin-naive patient tested prior to and between 2 to 30 h after aspirin (325 mg) ingestion (15), and is defined as aspirin-resistant. From this study, both the sensitivity (92%) and the specificity (85%) of this assay were determined. The coefficient of variance was 2.5% on repeated measures within patients. The between-patient coefficient of variance was 12.5% for baseline samples and 15.0% for post-aspirin samples. Digital angiograms were analyzed off-line using a computer-based edge-detection program (CMS-GFT, MEDIS, Leiden, The Netherlands) by experienced cardiologists who were unaware of the patient characteristics and outcomes.

Statistical analysis. Comparisons between the two groups were performed by the Mann-Whitney *U* test for continuous variables and by the Fisher exact test for dichotomous variables. A logistic regression analysis using forward technique was employed to determine significant independent predictors of CK-MB elevation. A significant level was defined when $p < 0.05$. All analyses were performed using SPSS 10.0 (SPSS Inc., Chicago, Illinois).

RESULTS

The clinical, angiographic, and procedural characteristics of the patients are listed in Tables 1 and 2. A total of 29 (19.2%) out of 151 enrolled patients were found to be aspirin-resistant. The characteristics were matched in the

Table 1. Baseline Clinical Characteristics

	Aspirin-Sensitive (n = 122)	Aspirin-Resistant (n = 29)	p Value
Age, yrs	63.7 ± 11.8	65.6 ± 9.7	0.426
Women, %	19.7	44.8	0.007
Weight, kg	67.2 ± 11.2	66.9 ± 12.9	0.901
Body mass index, kg/m²	25.4 ± 3.1	26.6 ± 3.9	0.099
Diabetes, %	40.2	44.8	0.678
Insulin-requiring	3.3	10.3	
Oral drugs	32.0	31.0	0.130
Diet	4.9	3.4	
Hypertension, %	77.0	75.9	1.000
Hyperlipidemia, %	77.9	72.4	0.625
Current smoker, %	12.3	17.2	0.542
Prior myocardial infarction, %	35.2	27.6	0.516
Total cholesterol, mmol/l	4.52 ± 1.28	4.08 ± 0.82	0.112
Creatinine, μmol/l	113 ± 67	123 ± 46	0.156
Renal insufficiency, %	11.5	24.1	0.130
Baseline medications			
Aspirin, mg	117 ± 49	103 ± 20	0.401
Aspirin 80 mg, %	18.9	17.2	
Aspirin 100–160 mg, %	76.2	82.8	0.322
Aspirin 300 mg, %	4.9	0	
Statin, %	82.8	79.3	0.788
Beta-blocker, %	74.6	82.8	0.469
ACE inhibitor, %	55.7	48.3	0.536
Calcium channel blocker, %	27.0	37.9	0.262
NSAID use 3 months to 2 weeks prior to PCI, %	1.6	3.4	0.475
Clopidogrel loading-to-device activation interval, h	24.6 ± 6.9	25.8 ± 12.2	0.697
Indication for PCI, %			0.492
Stable angina	72.1	79.3	
Unstable angina >2 weeks	10.7	3.4	
Myocardial infarction >2 weeks	17.2	17.2	

Body mass index is calculated as the weight in kilograms divided by the square of height in meters.
ACE = angiotensin-converting enzyme; NSAID = non-steroidal anti-inflammatory drugs; PCI = percutaneous coronary intervention.

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Table 2. Angiographic and Procedural Characteristics

	Aspirin-Sensitive (n = 122)	Aspirin-Resistant (n = 29)	p Value
Lesion location, %			0.223
LAD	36.8	47.7	
LCX	38.7	18.2	
RCA	22.1	29.5	
LM	2.5	4.5	
Reference vessel diameter, mm	2.66 ± 0.57	2.51 ± 0.55	0.121
Restenotic lesions, %	12.3	11.4	1.000
Thrombus present, %	2.5	0	1.000
Bifurcation lesion, %	27.9	44.8	0.117
AHA/ACC classification, %			0.543
A/B1	20.9	25.0	
B2/C	79.1	75.0	
Procedural anticoagulation			
Unfractionated heparin, %	91	100	0.124
Activated clotting time, s	369 ± 92	371 ± 98	0.918
No. of lesions treated	1.3 ± 0.5	1.5 ± 0.7	1.000
Stents placed, %	89.3	93.1	0.737
No. of stents per procedure	1.4 ± 0.8	1.6 ± 0.8	0.240
Maximal stent inflation pressure, atm	14.8 ± 3.4	15.1 ± 3.1	0.673
Rotational atherectomy, %	7.4	3.4	0.688
Transient vessel closure, %	0	3.4	0.192
Transient or persistent side branch closure, %	9.0	10.3	0.733

AHA/ACC = American Heart Association/American College of Cardiology; LAD = left anterior descending artery; LM = left main coronary artery; LCX = left circumflex artery; RCA = right coronary artery.

two groups except for the higher incidence of female patients (44.8% vs 19.7%; p = 0.007) in the aspirin-resistant group. All patients underwent successful PCI with <50% diameter stenosis at the target lesion(s) and Thrombolysis In Myocardial Infarction flow grade 3 after the intervention. There was no bailout use of glycoprotein IIb/IIIa inhibitors in any of the patients; no clinical bleeding events occurred. The ratios of hemoglobin pre- and post-PCI in the aspirin-sensitive and -resistant groups were identical at 0.96. None of the patients developed contrast nephropathy. Post-PCI myonecrosis occurred more frequently in the aspirin-resistant patients than in the aspirin-sensitive patients. The incidence of any CK-MB elevation was 51.7% versus 24.6% in the aspirin-resistant and -sensitive groups, respectively (p = 0.006) (Fig. 1). Elevation of TnI occurred in 65.5% of aspirin-resistant patients and 38.5% of aspirin-sensitive patients (p = 0.012) (Fig. 1). The median peak values of CK-MB and TnI in the aspirin-resistant and -sensitive groups, respectively, were 20 and 17 U/l and 6.3 and 0.85 ng/ml. The continuous relationship between ARU and

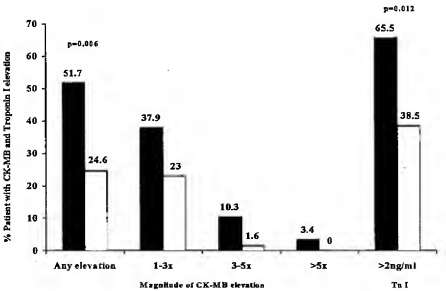

Figure 1. Incidence and magnitude of creatine kinase-myocardial band (CK-MB) and troponin I (TnI) elevation in aspirin-resistant (solid bars) and aspirin-sensitive (open bars) patients after percutaneous coronary intervention.

CK-MB, TnI, or bleeding index was not observed. No in-hospital mortality or urgent target vessel revascularization occurred among any of the patients. Variables associated with CK-MB elevation by univariate analysis were aspirin resistance ($p = 0.006$), bifurcation lesion ($p = 0.035$), B2/C lesion ($p = 0.029$), and number of stents used ($p = 0.04$). Multivariate analysis revealed aspirin resistance (odds ratio [OR] 2.9; 95% confidence interval [CI] 1.2 to 6.9; $p = 0.015$) and bifurcation lesion (OR 2.8; 95% CI 1.3 to 6.0; $p = 0.007$) to be independent predictors of CK-MB elevation after PCI.

DISCUSSION

This is the first study to demonstrate that, despite adequate pretreatment with clopidogrel, patients undergoing non-urgent PCI are at increased risk of myonecrosis when they are determined to be aspirin-resistant using a point-of-care assay, compared with those who are aspirin-sensitive. Elevation of CK-MB has been shown to be associated with a higher incidence of death, myocardial infarction, and repeat revascularization after PCI (16). Prevention of post-PCI myonecrosis, therefore, is of clinical importance. Aspirin has been shown to reduce acute thrombotic complications of balloon angioplasty (17–19). Thienopyridine pretreatment (2–7) and the addition of intravenous glycoprotein IIb/IIIa inhibitors (20–23) further improve the outcomes of patients undergoing elective or urgent PCI. However, the role of aspirin in PCI has not been defined in the contemporary era using double or triple antiplatelet therapy.

Aspirin resistance describes the clinical observation of the inability of aspirin to prevent thrombotic complications or the laboratory phenomenon of absence of the effect of aspirin on platelet inhibition tests. Four prior studies demonstrated the association of adverse clinical events in patients with aspirin resistance as determined by different assays (8,12–14). Our study extends these observations and provides further evidence on the clinical significance of aspirin resistance. We collected the data prospectively in stable patients undergoing PCI while interventionalists and laboratory personnel performing assays for myonecrosis were blinded to platelet inhibition results. The relatively high percentages of CK-MB and TnI elevations in our population may be due to the high rates of diabetes (≥40%) and complex lesions (≥75% B2/C lesions), and diffuse atherosclerosis as reflected by small reference diameters of ~2.6 mm. Despite receiving the maximal antiplatelet effect of clopidogrel with a 300-mg loading dose given >12 h before non-urgent PCI, aspirin-resistant patients had a 2.9-fold increased risk of CK-MB elevation compared with aspirin-sensitive patients.

Our study has several potential limitations. First, the study population was small and Asian in origin. Important trends may not be detected because of a lack of statistical significance and it is not known whether ethnicity plays a role in the differences in aspirin resistance. Second, our study did not have a prospective randomized design, and there might be unrecognized confounders that may influence the occurrence of myonecrosis in addition to aspirin responsiveness. Third, the antiplatelet effect of aspirin may fluctuate in patients at the same dosage. A single baseline measurement may not reflect the extent of platelet inhibition over long periods of time. However, during PCI when maximal antithrombotic action is desirable, a point-of-care platelet inhibition assay may give instant information on the efficacy of aspirin, regardless of previous antiplatelet effect.

Finally, we conclude that aspirin resistance is associated with a 2.9-fold increased incidence of myonecrosis as evidenced by CK-MB elevation following non-urgent PCI with adequate clopidogrel pretreatment. Our results may have implications for the need of identification of aspirin resistance in patients undergoing PCI and the use of alternative or additional antithrombotic therapy to minimize procedural complications.

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REFERENCES

1. Popma JJ, Weitz J, Bittl JA, et al. Antithrombotic therapy in patients undergoing coronary angioplasty. Chest 1998;114:728S–41S.
2. Steinhubl SR, Lauer MS, Mukherjee DP, et al. The duration of pretreatment with ticlopidine prior to stenting is associated with the risk of procedure-related non–Q-wave myocardial infarctions. J Am Coll Cardiol 1998;32:1366–70.
3. Steinhubl SR, Ellis SG, Wolski K, et al. Ticlopidine pretreatment before coronary stenting is associated with sustained decrease in adverse cardiac events: data from the Evaluation of Platelet IIb/IIIa Inhibitor for Stenting Trial (EPISTENT). Circulation 2001;103: 1403–9.
4. Assali AR, Salloum J, Sdringola S, et al. Effects of clopidogrel pretreatment before percutaneous coronary intervention in patients treated with glycoprotein IIb/IIIa inhibitors (abciximab or tirofiban). Am J Cardiol 2001;88:884–6.
5. Mehta SR, Yusuf S, Peters RJ, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet 2001;358:527–33.
6. Steinhubl SR, Berger PB, Mann JT 3rd, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA 2002;288:2411–20.
7. Chan AW, Moliterno DJ, Berger PB, et al. Triple antiplatelet therapy during percutaneous coronary intervention is associated with improved outcomes including one-year survival: results from the Do Tirofiban and ReoPro Give Similar Efficacy Outcome Trial (TARGET). J Am Coll Cardiol 2003;42:1188–95.
8. Grotemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non-responder. A pilot study including 180 post-stroke patients. Thromb Res 1993;71:397–403.
9. Helgason CM, Bolin KM, Hoff JA, et al. Development of aspirin resistance in persons with previous ischemic stroke. Stroke 1994;25: 2331–6.
10. Pappas JM, Westengard JC, Bull BS. Population variability in the effect of aspirin on platelet function. Implications for clinical trials and therapy. Arch Pathol Lab Med 1994;118:801–4.

11. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001;88:230–5.
12. Mueller MR, Salat A, Stangl P, et al. Variable platelet response to low-dose aspirin and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. Thromb Haemost 1997;78:1003–7.
13. Eikelboom JW, Hirsh J, Weitz JI, et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002;105:1650–5.
14. Gum PA, Kottke-Marchant K, Welsh PA, et al. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. J Am Coll Cardiol 2003;41:961–5.
15. Ultegra Rapid Platelet Function Assay-ASA (RPFA-ASA) [package insert]. San Diego, CA: Accumetrics Inc., 2002.
16. Califf RM, Abdelmeguid AE, Kuntz RE, et al. Myonecrosis after revascularization procedures. J Am Coll Cardiol 1998;31:241–51.
17. Barnathan ES, Schwartz JS, Taylor L, et al. Aspirin and dipyridamole in the prevention of acute coronary thrombosis complicating coronary angioplasty. Circulation 1987;76:125–34.
18. Schwartz L, Bourassa MG, Lesperance J, et al. Aspirin and dipyridamole in the prevention of restenosis after percutaneous transluminal coronary angioplasty. N Engl J Med 1988;318:1714–9.
19. Lembo NJ, Black AJR, Roubin GS, et al. Effect of pre-treatment with aspirin versus aspirin plus dipyridamole on frequency and type of acute complications of percutaneous transluminal coronary angioplasty. Am J Cardiol 1990;65:422–6.
20. The EPIC Investigators. Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. N Engl J Med 1994;330:956–61.
21. The EPILOG Investigators. Platelet glycoprotein IIb/IIIa receptor blockade and low-dose heparin during percutaneous coronary revascularization. N Engl J Med 1997;336:1689–96.
22. The EPISTENT Investigators. Randomised, placebo-controlled and balloon angioplasty-controlled trial to assess safety of coronary stenting with the use of platelet glycoprotein IIb/IIIa blockade. Lancet 1998; 352:87–92.
23. The ESPRIT Investigators. Novel dosing regimen of eptifibatide in planned coronary stent implantation (ESPRIT): a randomised, placebo-controlled trial. Lancet 2000;356:2037–44.

Exhibit D

European Medicines Agency
Evaluation of Medicines for Human Use

Doc.Ref.: EMEA/117561/2009

ASSESSMENT REPORT

FOR

Efient

International Nonproprietary Name: prasugrel

Procedure No. EMEA/H/C/000984

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Eli Lilly Nederland B.V. submitted on 06 February 2008 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for Efient, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMEA/CHMP on 25 January 2007.

The legal basis for this application refers to:

A - Centralised / Article 8(3) / New active substance.

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

The application submitted is a complete dossier composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Licensing status:
The product was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur:	Steffen Thirstrup	Co-Rapporteur:	Gonzalo Calvo Rojas

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 6 February 2008.
- The procedure started on 27 February 2008.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 19 May 2008. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 20 May 2008.
- During the meeting 23-26 June 2008, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 26 June 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 21 July 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 9 September 2008.
- During the CHMP meeting on 22-25 September 2008, the CHMP agreed on a List of Outstanding Issues to be addressed in writing and in an oral explanation by the applicant and in addition CHMP agreed on questions to be addressed to a SAG-CVS.
- The applicant submitted the written responses to the CHMP List of Outstanding Issues on 16 October 2008.
- During a meeting of a SAG group on 30 October 2008, experts were convened to address questions raised by the CHMP.
- During the CHMP meeting on 17-20 November 2008, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 15-18 December 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Efient on 18 December 2008. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 16 December 2008.

- The CHMP opinions were forwarded in all official languages of the European Union, to the European Commission, which adopted the corresponding Decision on 25 February 2009.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

Platelets play a central role in the pathogenesis of atherothrombosis and in the formation of thrombi following coronary angioplasty, with and without stent implantation. Platelets initially adhere at sites of vascular injury, atherosclerotic plaque rupture, balloon angioplasty, and stenting. Platelet activation following these interactions results in the release of ADP, thromboxane A2, and other mediators. Released ADP promotes platelet activation via the G-protein linked P2Y1 and P2Y12 purinergic receptors leading to further platelet activation, aggregation, and other platelet functions, such as platelet shape change, secretion, and the development of pro-coagulant and pro-inflammatory activities.

Activated platelets are recruited to sites of coronary plaque rupture and intra-arterial stenting, thereby forming aggregates that may lead to platelet-rich thrombi, vascular occlusion, tissue ischemia, and myocardial necrosis in what is collectively known as Acute Coronary Syndrome (ACS). The term ACS is a pathophysiological continuum progressing from ischemic chest pain with sudden onset and worsening (UA), to ischemia severe enough to cause irreversible myocardial damage detected with cardiac biomarkers without persistent ST-segment elevation (NSTEMI), to total occlusion of the culprit coronary artery with persistent ST-segment elevation, resulting in myocardial necrosis and elevated biomarkers (STEMI).

ACS occurs in a diverse global population and has a significant socioeconomic impact as patients require hospitalization, rehabilitation, and often suffer subsequent ischemic events.

Acute coronary syndromes will likely remain one of the leading causes of hospitalisation worldwide due to the increasing prevalence of risk factors for coronary heart disease and the increasing size of the aged population.

Options for the initial management of ACS include pharmacotherapy alone or an early invasive strategy with PCI (with or without coronary stenting) or coronary artery bypass grafting (CABG) as guided by the results of coronary angiography. The current American College of Cardiology/American Heart Association (ACC/AHA) and European Society of Cardiology (ESC) guidelines recommend an early invasive strategy for ACS patients with intermediate to high-risk features. Pharmacotherapy includes both anticoagulant and anti-platelet drugs. The current standard of care for patients with ACS includes dual anti-platelet therapy with aspirin and thienopyridine in both the acute and chronic phases of treatment. This therapy improves outcome in patients with ACS and those undergoing percutaneus coronary intervention (PCI); the high risk of for early stent-associated thrombosis is substantially reduced by dual antiplatelet therapy. Ticlopidine and clopidogrel are the two currently approved thienopyridines. They are pro-drugs requiring in vivo metabolism to form the active metabolite that binds rapidly and irreversibly to platelet $P2Y_{12}$ receptors, thus inhibiting platelet aggregation mediated by the $P2Y_{12}$ receptor. Clopidogrel has largely replaced ticlopidine due to its once-daily dosing regimen, improved tolerability and lowered incidence of adverse hematolgical side effects.

Several potential limitations of clopidogrel therapy have been identified despite loading dose of clopidogrel. This includes marked inter-individual variability in platelet inhibition and relatively slow onset of action. An association between thrombotic complications following PCI and poor antiplatelet response to the approved standard clopidogrel dosing regimen (loading dose (LD) 300 mg and maintenance dose (MD) 75 mg) has been suggested. Further, it has been shown that "non-responsiveness" to a clopidogrel 600 mg LD is a strong predictor of stent thrombosis in patients receiving drug-eluting stents, and in addition, that residual platelet aggregation above the median is associated with a 6.7-fold increased risk of major adverse cardiac events (death, myocardial infarction and target vessel revascularisation) at 1 month follow-up in patients undergoing elective PCI.

These observations suggest the possibility that higher and more consistent levels of platelet inhibition may improve clinical outcome in patients with ACS undergoing PCI.

Prasugrel, a thienopyridine adenosine diphosphate (ADP) receptor antagonist, is an orally administered pro-drug requiring in vivo metabolism to form the active metabolite (R-138727) that

irreversibly inhibits platelet activation and aggregation mediated by the $P2Y_{12}$-receptor. Prasugrel has a distinct chemical structure that permits efficient conversion to its active metabolite through rapid hydrolysis by carboxylesterases and then by multiple cytochrome P450 (CYP) enzymes. Once bound, a platelet is inhibited for its remaining lifespan. After prasugrel dosing is stopped, a return to baseline levels of platelet aggregation will occur as new platelets are formed. The return to baseline typically occurs over about 7 to 10 days after treatment is stopped.
Non-clinical studies indicated that, with respect to inhibiting ex vivo platelet aggregation and in vivo thrombus formation, prasugrel was approximately 10-100-fold more potent than clopidogrel and ticlopidine, respectively. Early clinical data in healthy subjects confirmed the greater platelet inhibition and more consistent response to prasugrel compared to clopidogrel. While the active metabolites of prasugrel and clopidogrel resulted in similar levels of platelet inhibition in vitro, the amount of each active metabolite generated in vivo was quite different, with prasugrel LD (60 mg) resulting in approximately 50-fold greater exposure, on pr. Mg basis, to its active metabolite compared to clopidogrel LD of 300 mg. This observation provides a mechanistic basis for the faster, higher and more consistent inhibition of platelet aggregation (IPA) observed with prasugrel.

2.2 Quality aspects

Introduction

Efient contains prasugrel hydrochloride as active substance. Prasugrel is a member of the thienopyridine class of antiplatelet agents. Currently available thienopyridines include clopidogrel and ticlopidine. Prasugrel is an orally bioavailable prodrug metabolized to an active adenosine diphosphate (ADP) receptor antagonist, which is a potent inhibitor of platelet activation and aggregation mediated by the $P2Y_{12}$ ADP receptor.
Efient is an immediate release, double-arrow shaped, film-coated, debossed tablet. Tablets contain either 5 or 10 mg of prasugrel and different strengths are differentiated by size, film-coating colour and debossing. The tablets are commercially supplied in blister packaging.

Active Substance

The INN name of the active substance is prasugrel which is present in the product in the form of the hydrochloride salt. The chemical name is 5-[(1*RS*)-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate hydrochloride corresponding to the molecular formula C20H20FNO3S•HCl and molecular mass of 409.90
Prasugrel hydrochloride is white to light brown crystalline solid, slightly hygroscopic and soluble to slightly soluble at pH 1-4, very slightly soluble at pH 5 and practically insoluble at pH 6-7. The pKa value of prasugrel hydrochloride was 5.1. It shows polymorphism. It is obtained as a racemic mixture; therefore, it shows no optical rotation.

Prasugrel hydrochloride is a prodrug. In aqueous media, cleavage of the ester moiety forms the hydrolysis product, which exists as a mixture of diastereomers, and which are the precursors of the active metabolite. The hydrochloride is used because of its better hydrolytic stability and because it provides a better solubility at relevant physiological pHs.

- Manufacture

The synthetic route involves 3 steps where production of an intermediate, production of prasugrel free base and production of prasugrel hydrochloride consecutively takes place.
. In-process controls performed are suitable to control the reaction progress. The starting materials are considered simple molecules and satisfactory specifications were presented.
During development of the drug substance manufacturing process, quality attributes of the drug substance were evaluated with respect to the drug product manufacturing process and with respect to their impact on the critical quality attributes of the drug product. This analysis resulted in the identification of six drug substance critical quality attributes. The drug substance specification has been established to confirm that the manufacturing process reproducibly and reliably produces a drug substance that meets the critical quality attributes. Potential critical process parameters (CPPs) were identified by statistical design methods and a mechanistic understanding of the drug substance process.

All manufacturing steps are thoroughly examined and design spaces have been developed.
Concerning the use of concentrated HCl, there is a potential risk that acetone is converted into diacetone alcohol and then to mesityl oxide. However, any level in the final substance is below the LOQ, which is below the limit of toxicological concern.
The same synthetic route has been used to prepare all of the prasugrel hydrochloride salt used in clinical and development studies and it is the synthetic route for commercial drug substance manufacture.

- Specification

The drug substance specification includes tests for appearance (visual), identification (IR for prasugrel selective precipitation for Cl), assay (HPLC), impurities (HPLC), residual solvents (GC), water (Karl Fischer), Fineness (sieving) and Specific Surface Area (BET).

Results for 3 commercial scale batches were provided analyzed by the current analytical methods and against the current specifications. The results comply with the specification.
In addition, results of another numerous historical batches were provided as supportive data. However these batches were tested by analytical methodologies and against specifications that both have evolved during development.

- Stability

Three pilot batches (50% of full scale) were put on long-term (25°C/ 60%RH) and accelerated (40°C/ 75%RH) stability testing conditions respectively. In addition results from supporting stability studies were presented on another three earlier batches manufactured at both pilot and full scale. However the use of different equipment in the manufacture of the pilot and early batches resulted in differences in the chemical stability of the active substance and therefore the equipment yielding to more stable material was chosen. All these batches have been stored in the proposed market packaging with the exception of the supporting stability batches where desiccant was not included. 24 months of stability data were available at the long-term storage condition of 25°C/60% RH for the primary stability studies, up to 36 months for the supporting stability studies and six months data under accelerated conditions.
It was apparent from the results that generally no significant changes are seen neither at 25°C/60% RH nor at 40 °C/ 75 %RH except for an impurity which increased, but still within the limit.

The photostability of prasugrel hydrochloride in the solid state was assessed and results showed that it does not need to be protected from light in the solid state.
Finally stress testing studies have been conducted on prasugrel hydrochloride drug substance in order to gain an understanding of its degradation chemistry
The conclusion from the stress degradation, long term, and accelerated stability studies is that prasugrel hydrochloride drug substance is stable when packaged in the container closure system proposed. Prasugrel hydrochloride is susceptible to hydrolysis and therefore contact with water should be avoided. The results of these primary stability studies demonstrate that the drug substance is stable when stored at room temperature in the appropriate packaging system. The data collected to date support the proposed retest period.

Medicinal Product

- Pharmaceutical Development

Prasugrel hydrochloride is a prodrug. Initial clinical studies were conducted using prasugrel free base tablets. However, prasugrel hydrochloride was selected for commercial development based on the higher solubility of this salt form relative to the free base across the gastrointestinal pH range. Initial trials however exhibited undesirable degradation product formation and demonstrated that the hydrochloride salt was more susceptible to hydrolysis than the free base.

Nevertheless the use of the salt rather than the free base was selected as a result of clinical data indicating that the rate and/or extent of absorption of the free base is adversely affected if the patient takes concomitant medications, which increase gastric pH. Above pH 6, the bioavailability of prasugrel free base was substantially reduced. Based on these results, it was decided to develop the

prasugrel hydrochloride tablet formulations. Solubility determination results and permeability and metabolism information suggest that prasugrel HCl is a BCS class 2 compound.
Also, a food effect study and a study with a gastric pH modifier were conducted in humans to assess the in vivo performance of prasugrel hydrochloride or prasugrel free base tablets.
As prasugrel hydrochloride is susceptible to both hydrolytic and oxidative degradation, the formulation, manufacturing process, and packaging of tablets focused on approaches to maintain product stability.
An extensive formulation development has been conducted. Design spaces have been defined through statistically-designed and individual studies. Critical and non-critical process parameters have been defined. A finished product specification covering all normal parameters has been set up. The quality features are provided in prasugrel hydrochloride tablets using the concepts and elements of Quality by Design with risk assessment and risk mitigation in order to ensure that key product attributes were defined at an early stage.
The choice and function of the excipients in the formulation was based on the need for excipients that have the smallest possible impact on the degradation of the drug substance in formulation and the physical properties necessary for the manufacturing process.
During development, core tablet strengths ranging from 5 mg to 15 mg were developed that are qualitatively identical and quantitatively vary only in the percent w/w drug loading with concomitant adjustment of the diluent, the percentage of the other excipients in the core tablet are identical. A standard film coating is applied to produce tablets of uniform colour.
A number of studies were conducted to determine formulation robustness of the process and the formulation. Dissolution is affected by pH and decreases with increasing pH.
Clinical studies have demonstrated that tablet performance is not affected by formation of the free base over a range of 5%-70% conversion. AUC and C_{max} of the active metabolite were bioequivalent after 1 hour.
A reaction between prasugrel HCl and an excipient was observed late in the development studies during manufacture and storage. This reaction leads to a partial and irreversible formation of prasugrel free base in the tablets. Analysing the samples used for clinical phase 3 study indicated that salt-to-base formation of at least up to 70% had no clinical impact and a requirement has been included in the finished product specification.

Due to prasugrel hydrochloride susceptibility to hydrolytic and oxidative degradation a dry manufacturing process was selected. Extensive experiments have been conducted to ensure a robust manufacturing process through design spaces. This has been used to set up the process controls for the production batches.. The container has been chosen to minimize humidity and to provide the necessary oxygen protection through out the shelf life of the product.

- Adventitious Agents

None of the excipients are animal-sourced, thus eliminating any risk of TSE contamination in the tablet formulation. The film-coating colour mixture utilizes a single animal-sourced excipient, lactose monohydrate. The source of the lactose complies with regulations to ensure patient safety.

- Manufacture of the Product

A dry manufacturing process is utilised for the manufacture of Efient comprising the following steps: blending, dry granulation, blending, compression, coating and drying of tablets, packaging. The manufacturing process is sufficiently described and in-process controls are adequate.
Validation data on three commercial-scale 5 mg batches and three commercial-scale 10 mg batches provided satisfactory reassurance for the reproducibility and consistency of the manufacturing process.

- Product Specification

The specifications of the drug product at release and shelf-life include tests for appearance (visual), identity (IR), assay (HPLC), uniformity of dosage units (Ph.Eur.), degradation products (HPLC), dye identity test (not routinely), dissolution (Ph.Eur.), tablet form conversion (XRPD).
Batch results are provided for commercial scale batches and clinical trials batches. The results comply with the specification, confirm consistency of the product and support the acceptance criteria.

- Stability of the Product

Stability studies have been conducted according to ICH guidances.
Three production scale batches of 5 mg tablets have been stored at 25°C/60% RH for 12 months, at 30°C/75% RH, for 12 months and at 40°C/75% RH for 6 months in the proposed market packaging.
Another three production scale batches of 10 mg tablets have been stored at 25°C/60% RH for 18 months, at 30°C/65% RH, for 18 months and at 40°C/75% RH for 6 months in the proposed market packaging.
Bulk simulator samples of both 5 and 10 mg tablets were also stored at 25°C/60% RH and at 5°C for 12 months, at 40°C/75% RH for 1 month and at -20°C for 1 month.
Additionally a supporting study for the 10 mg tablets stored in blisters was presented.
Stability results indicate that all tested parameters remain within the specification limits. Degradation products levels tend to increase but comply with the individual specification requirements at long term conditions throughout 24 months (statistically).

Photostability: A production scale batch of each strength was tested according to ICHQ1B. It is concluded from the results that no special precautions are required since the blister provides the necessary protection and the product is to be labelled to be kept in the original package.

Stress testing: A production scale batch of each strength was used for stress testing together with a placebo.. It was found that the prasugrel in the tablets degraded with exposure to heat and moisture, particularly in an ambient oxygen environment. Prasugrel does not degrade significantly with exposure to simulated sunlight.

Tablet Form Conversion: Primary stability samples were analyzed for the level of prasugrel free base after storage in both bulk and commercial packages. The conversion of prasugrel hydrochloride to prasugrel free base is primarily due to exposure to moisture. . However the present manufacturing method is shown to provide the necessary protection against moisture.

Discussion on chemical, pharmaceutical and biological aspects
The quality of Efient film-coated tablet is adequately established. Information on development, manufacture and control of the drug substance has been presented in a satisfactory manner. The quality of the active substance is considered sufficiently described and adequately supported by data. Sufficient chemical and pharmaceutical documentation relating to development, manufacture and control of the drug product has been presented. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance.
Stability tests indicate that the product under ICH guidelines conditions is chemically stable for the proposed shelf life.

2.3 Non-clinical aspects

Introduction

Prasugrel belongs to the thienopyridine class of prodrugs and is inactive *in vitro*. Initial studies required dosing animals with prasugrel with subsequent blood collection to look for *in vivo* activation as reflected in *ex vivo* pharmacodynamics measurements. Once the *ex vivo* evidence for the activation of prasugrel *in vivo* was established, subsequent studies addressed the potential activity of prasugrel in disease models of target indications (thrombosis). Prasugrel administration resulted in prolongation of the bleeding time as did clopidogrel and ticlopidine as it was seen in a model of haemostasis.

Consistent with differing mechanisms of action, co-administration of aspirin showed additive/synergistic interaction in studies of both thrombosis and haemostasis. Having established the *in vivo* activity of prasugrel in disease models reflecting the clinical target indication, studies were performed to characterize the activities of the active metabolite of prasugrel by *in vitro* studies.

The rationale for the non clinical development and application for marketing approval of prasugrel is considered well established. The extent and scope of the documentation provided are appropriate to characterise the non clinical profile of the product.

The following guidelines were considered: Note for guidance on safety pharmacology studies for human pharmaceuticals (CPMP/ICH/539/00), Note for Guidance on Toxicokinetics: the assessment of systemic exposure in toxicity studies (CPMP/ICH/384/95), Non-clinical guideline on drug-induced hepatotoxicity (CHMP/SWP/150115/2006), Note for Guidance on carcinogenicity: testing for carcinogenicity of pharmaceuticals (CPMP/ICH/299/95), Note for Guidance on the detection of toxicity to reproduction for medicinal products and toxicity to male fertility (CPMP/ICH/386/95), Guideline on risk assessment of medicinal products on human reproduction and lactation: from data to labelling (EMEA/CHMP/203927/05), and Guidance on the Environmental risk assessment of medicinal products for human use (CHMP/SWP/4447/00).

The analytical method validation study in beagle dogs for the pharmacokinetics of prasugrel active metabolite was conducted in compliance with the GLP guidelines.

The safety pharmacology studies provide an evaluation of the safety pharmacology of prasugrel and meet the standards for general pharmacology studies in effect at the time of their conduct. This is considered acceptable by the CHMP. All pivotal toxicity studies were conducted in compliance with GLP regulations.

Pharmacology

- Primary pharmacodynamics

In *ex vivo* studies with rats, dogs, and cynomolgus monkeys, prasugrel demonstrated dose-dependent inhibition of ADP-induced platelet aggregation. Unless indicated otherwise, platelet function studies were performed using light transmission aggregometry (LTA), which monitors the increase in light transmission in stirred suspensions of platelets in citrated plasma (platelet-rich plasma, PRP) as they aggregate in response to activation with agonists such as ADP. ADP is a natural ligand for the target receptor ($P2Y_{12}$) of the thienopyridine class of oral antiplatelet agents (ticlopidine, clopidogrel, and prasugrel).

The selectivity of prasugrel for antagonism of ADP-induced platelet aggregation was demonstrated by the lesser inhibition of aggregation achieved with thrombin *vs* ADP in platelets under *ex vivo* conditions. Prasugrel's inhibitory effects were maintained after washing of the platelets, showing an irreversible platelet inhibition. Studies in rats compared prasugrel's potency with that of clopidogrel and indicated a faster onset of action, since prasugrel (1-10 mg/kg, p.o.) caused dose-dependent inhibition of platelet aggregation at 0.5 hr after dosing with an ED_{50} value of 4.2 mg/kg, suggesting an early onset of action. In contrast, clopidogrel (10-100 mg/kg, p.o) showed moderate effect at 0.5 hr (ED_{50} > 100 mg/kg). The maximum effect of both prasugrel and clopidogrel were observed at 4 hr after administration; the ED_{50} values being 1.1 mg/kg (p.o.) and 15 mg/kg (p.o.), for prasugrel and clopidogrel, respectively. The inhibitory effects of prasugrel (1- 3 mg/kg) and clopidogrel (10-30 mg/kg) were long-lasting, and these inhibitions completely disappeared at 96 hr after administration.

The *ex vivo* effects of prasugrel on platelet aggregation in male cynomolgus monkeys assessed as the ADP (10μM)-induced platelet aggregation in platelet-rich before and after oral administration of prasugrel showed that prasugrel (0.1 and 0.3 mg/kg/day) given orally once a day for 14 days inhibited platelet aggregation in a dose-dependent manner. The inhibitory effect reached a plateau on days 3 to 5, suggesting cumulative effects of prasugrel, and was maintained during the administration of prasugrel after reaching the maximal effect. The effects slowly declined after cessation of prasugrel administration. There were no significant inhibitions of platelet aggregation on the 7th day after the final dose of prasugrel (day 21). These results indicate that repeatedly administered prasugrel exhibits a potent and long-lasting antiplatelet effect.

Inhibitory effects of 14 day lasting repeated administration of prasugrel (0.03-0.3 mg/kg/day, p.o.) on platelet aggregation in the beagle dog were investigated. The ADP (8μM)-induced platelet aggregation

measurements showed inhibitory effects of prasugrel (0.1 and 0.3 mg/kg/day) reaching plateau on day 3. After cessation of administration, inhibition of platelet aggregation gradually decreased.

The *in vivo* effects of prasugrel were assessed in various non clinical pathophysiological models of thrombotic challenge:

- The arterio-venous shunt model
- The electrical injury model
- The stroke model
- The pathophysiological model of peripheral artery disease
- The bleeding time model

In the rat arterio-venous shunt model, prasugrel reduced thrombus formation in a dose-dependent manner. Similarly, prasugrel prolonged the time to occlusion and increased the patency in the electrical injury model of arterial thrombosis in a dose-dependent way. The cumulative inhibitory nature of repeat dosing with thienopyridines was demonstrated using the same model during a repeated 3 day dosing regimen. Treatment with prasugrel resulted in a dose-dependent reduction of the incidence, total area, and the number of cerebral infarcts in a model of embolic cerebral infarction in the rat, while clopidogrel had lower activity. In a model of peripheral arterial disease whereby injection of lauric acid into the rat femoral artery produces endothelial injury, platelet adhesion, and platelet aggregation, prasugrel dose-dependently inhibited progression of the lesions. Prasugrel also caused a prolongation of bleeding time in a tail transaction model in rat.

Prasugrel contains a chiral centre and thus, exists as two individual enantiomers: the R-enantiomer (R-96875) and the S-enantiomer (R-96876). The platelet inhibitory effects of the individual enantiomers were evaluated following the oral administration to both, rats and monkeys, and following single oral administration of the prasugrel's individual enantiomers to beagle dogs. Additional in vitro studies were conducted in order to evaluate the effects on platelet aggregation.

Oral administration of R-96875 and R-96876 (both at 1 and 3 mg/kg) to rats dose-dependently inhibited platelet aggregation at 2 and 4 hr after dosing, respectively. There were no statistically significant differences in the efficacy between R-96875 and R-96876 at the same dosage. The ED_{50} values at 4 hr after dosing were 1.4 mg/kg for R-96875 and 1.3 mg/kg for R-96876. Effects of a 3 day repeated administration of R-96875 and R-96876 (both at 0.3 mg/kg/day, p.o.) on ADP-induced platelet aggregation using platelet-rich plasma were also examined in cynomolgus monkeys. The wash-out periods between the two treatments was considered acceptable. Both isomers caused inhibition of platelet aggregation on day 3, and this effect was almost equal between the groups. There were no statistically significant differences in the efficacy between R-96875 and R-96876 at any point. These results indicate that oral administrations of optical isomers of prasugrel, R- 96875 and R-96876, exert a similar extent of *ex vivo* effect on platelet aggregation in rats and in cynomolgus monkeys.

The active metabolite R-138727 has two chiral centres, resulting in four enantiomers. Metabolite R-138727 has potent and selective $P2Y_{12}$ antagonistic activity. The two most potent enantiomers of R-138727, the R-125690 and R-125689, are about 100- and 10-fold more potent than enantiomers R-125687 and R-125688, respectively. The two most potent enantiomers comprised the majority of the circulating R-138727 in rats and humans.

In pharmacodynamic mechanistic studies, the active metabolite of prasugrel, R-99224, affected $P2Y_{12}$-specific biomarkers, including alpha granule release, fibrinogen binding, and restoration of ADP-induced reduction of PGE_1- induced elevation cAMP. In contrast, $P2Y_1$ biomarkers (platelet shape change, Ca^{2+} mobilisation) were unaffected by pre-incubation of platelets with prasugrel's active metabolite. This confirms that the inhibition of the platelet aggregation by prasugrel is mediated by $P2Y_{12}$ receptors. The pharmacological effects are most probably dependant on the production of the active metabolites of prasugrel.

Inhibitory effects of orally administered optical isomers of prasugrel on platelet aggregation investigated in beagle dogs was measured as the platelet aggregation induced by ADP (8 μM) at 2 and 4 hr post dosing. There were no significant differences in baseline values of platelet aggregation

among all groups. In the control group, there were no obvious changes in aggregation after vehicle administration. In contrast, each isomer (0.1-1 mg/kg, p.o.) inhibited platelet aggregation in a dose-dependent manner. There were no statistically significant differences in platelet aggregation at 2 and 4 hr post dose between the two isomers at corresponding doses. In addition, ED_{50} values for the two isomers were similar at 2 and 4 hr post dose. These results show that an oral administration of the optical isomers of prasugrel produces anti-platelet effects of similar potency in beagle dogs. This pharmacodynamic study supports the use of racemic prasugrel, since all four enantiomers are formed as was shown in a pharmacokinetic study in dogs.

In general, the *ex vivo* studies with rats, dogs, and cynomolgus monkeys demonstrated dose-dependent inhibition of ADP-induced platelet aggregation by prasugrel. Studies in rats also demonstrated prasugrel's potency compared to clopidogrel and suggested a faster onset of action. The selectivity of prasugrel antagonism of ADP-induced platelet aggregation was demonstrated by the lesser inhibition of aggregation achieved with thrombin *vs* ADP in platelets *ex vivo*. The inhibitory effect was maintained after washing of the platelets, showing an irreversible platelet inhibition.

- Secondary pharmacodynamics

No secondary pharmacodynamic studies were made on binding and activity to other proteins than $P2Y_{12}$ and $P2Y_1$ and the secondary pharmacology data were derived from the results of the safety pharmacology studies. The effects observed in these *in vitro* safety studies occur at a concentration at least more than 10-fold higher than the maximum therapeutic concentration observed in humans. Both, the non clinical *in vivo* studies and clinical studies, did not provide any evidence for unexpected secondary pharmacodynamic effects of prasugrel. Thus, further studies are deemed unnecessary. Nevertheless, the results of a screening of prasugrel and its metabolite M1 in a standard battery of receptor binding assays were requested by the CHMP. Thus, M1 and prasugrel were tested in a battery of receptor binding assays. Neither prasugrel nor M1 showed affinity for the tested receptor at concentrations up to 10 µM.

- Safety pharmacology programme

Assessments of *in vivo* activity of prasugrel included evaluation of cardiovascular, central nervous system (CNS), respiratory, renal, and gastrointestinal (GI) functioning in rodents or dogs.

Effects on the GI and CNS occurred at high doses of prasugrel. At an oral dose of 100 mg/kg, prasugrel produced a significant decrease in paradoxical sleep in rats, without altering the total percentage of time spent sleeping. Increased sensitivity to touch was observed in rats at the 300 mg/kg oral dose. Other CNS endpoints, including body temperature, clinical observations, precipitated seizure thresholds, spontaneous activity, and thiopental-induced sleep times, were not altered following administration of single oral doses of prasugrel up to 300 mg/kg. Examination of the effects on autonomic nervous system and smooth muscle showed that prasugrel inhibited spontaneous movement of isolated rabbit ileum at $1x10^{-4}$ g/ml and inhibited the amplitude and increased frequency of spontaneous motility of isolated pregnant rat uterus. Prasugrel at $1x10^{-5}$ g/ml significantly inhibited acetylcholine-, histamine- and serotonin induced contractions in isolated guinea pig ileum.

The potential for prasugrel to inhibit cardiac repolarisation was evaluated by examining the effect of three prasugrel metabolites on potassium currents in hERG-transfected cells. The metabolites R-138727 and R-106583 were evaluated because these are the active and the most abundant inactive human metabolites, respectively, and R-95913 was evaluated because it is the intermediary step between prasugrel and the active metabolite. No significant effects on the potassium currents in hERG-transfected CHO-K1 cells were observed at up to the highest concentrations tested (30 µM for R106583 and R138727; 15 µM for R-95913) which were greater than approximately 485 times the expected free C_{max} values of the three metabolites following a clinical loading dose of 60 mg prasugrel. Therefore, the hERG data for prasugrel metabolites do not suggest a potential impact of prasugrel on cardiac repolarisation due to inhibition of potassium currents. Prasugrel (30 and 100 mg/kg, ID) showed no major effects on heart rate, blood pressure, respiration rate, carotid blood flow, or pressure response to acetylcholine, norepinephrine or bilateral carotid occlusion in the anesthetised dogs. No effects on QT interval were observed in quantitative electrocardiograms evaluated in the 3 and 9

month repeat dose studies in dogs at doses approximately nine times the 60 mg clinical loading dose calculated as mg/m^2.

Prasugrel produced a decreased gastric acid content and gastric volume at 100 mg/kg in rats. Furthermore, prasugrel decreased gastric emptying in mice when given for 3 days at the dose of 300 mg/kg. However, the doses at which these effects occurred were ≥14 times the 60 mg clinical loading dose calculated as mg/m^2. Prasugrel (10-100 mg/kg, p.o.) had no effects on urinary volume, excretion of electrolytes or osmotic pressure in rats.

- Pharmacodynamic drug interactions

Thienopyridine antiplatelet agents are commonly used in combination with aspirin as "dual antiplatelet therapy". The use of the combination is based on the alternative receptor/signalling pathways that each of these agents inhibits and the additive, or synergistic, platelet inhibitory effects that results from co-administration. Pharmacodynamic studies were conducted with the combination of prasugrel/aspirin. An additional study involved co-administration of other drugs, in which the comparisons of the pharmacokinetics and pharmacodynamics of prasugrel base and hydrochloride salt were made in the presence of the proton pump inhibitor lansoprazole.

The additive activity of prasugrel and aspirin has been demonstrated in several studies of platelet aggregation (*ex vivo*) in rats and dogs, thrombus formation (*in vivo*) in rats, and bleeding time in rats. Consistent with these findings, *in vitro* studies with blood from human volunteers demonstrated that a combination of R-138727 and aspirin has additive effects on collagen-induced platelet aggregation.

The antiplatelet effects of two tablet formulations of prasugrel, the free base tablet and hydrochloride salt tablet, were compared in beagle dogs pretreated with lansoprazole, a proton pump inhibitor. Plasma concentrations of prasugrel metabolites at 1 hr post dosing were not significantly different from those of the free base tablet and hydrochloride salt tablet given to dogs. These results suggest that the free base tablet and hydrochloride salt tablet have similar antiplatelet potency in lansoprazole-treated dogs.

Pharmacokinetics

Absorption, distribution, metabolism, and excretion profile of prasugrel was investigated in mice, rats, and dogs, which are also the species used in the toxicological evaluation of the compound. Analytical methodology evolved adequately. In initial pharmacokinetic and absorption studies, some inactive metabolites of prasugrel were measured and their pharmacokinetic parameters used as indicators of the absorption and metabolism of prasugrel. A number of new metabolites were quantified and a method for determination of prasugrel's active metabolite concentrations in plasma was ultimately developed. Most studies were conducted following oral administration, the intended clinical route of administration.

Prasugrel is rapidly absorbed in all species including humans; T_{max} of the active metabolite R-138727 is less than 1 hour. Prasugrel itself was not detected in plasma after oral administration. The decline of prasugrel related radioactivity was biphasic in rats and dogs. The radioactivity terminal elimination half-life seemed to be similar in mice and rats, approximately 24 h, but it is considerably longer in dogs, approximately 3 days. In humans, the average terminal elimination half-life of the active metabolite R-138727 was approximately 7 hours. Approximately 21% of a [^{14}C]-prasugrel dose is excreted in human faeces within 48 hours. The pharmacokinetic studies have only been conducted in male animals. However, no apparent sex differences were observed during the repeat-dose toxicity studies. Following single oral doses of prasugrel base or prasugrel hydrochloride, the exposure to prasugrel metabolites was evaluated in the mouse, rat, and dog. Exposure parameters to prasugrel metabolites were higher for prasugrel hydrochloride compared with prasugrel base at doses of ≥500 mg/kg in the rat and at 100 mg/kg in the dog. Tissue distribution of radioactivity related to prasugrel was studied in rats following single and repeated oral administration. Radioactivity was widely and rapidly distributed throughout the body. The radioactivity concentration was highest in most tissues involved in the absorption and elimination of the compound and its metabolites, i.e., stomach, intestines, liver, kidney and urinary bladder. Prasugrel distributed to the bone marrow of rats with a

tissue-to-plasma ratio of less than 0.5. Following repeated daily dosing, accumulation consistent with the elimination half-life of prasugrel was observed in most organs. After a single oral dose of 5 mg/kg ^{14}C-prasugrel to rats on Day 13 of pregnancy, the fetal concentration of prasugrel radioactivity was 0.27 times that in maternal blood 1 hour after administration and declined thereafter, suggesting low placental transfer of prasugrel or its metabolites. Due to instability of the active metabolites R-138727 in plasma, the protein binding was only investigated in human serum albumin, where the metabolite was highly bound by 98% and the species differences in protein binding of the active metabolites R-138727 were not assessed. The protein binding of the inactive metabolites R-100932, R-106583 and R-95913 was similar in rats, dogs and humans (>80%) while the protein binding of the inactive metabolite R-119251 was significantly lower in dogs (26-36%) as compared to rats (71-77%) and humans (76-77%). Prasugrel was extensively metabolised in all species. A total of eighteen metabolites were identified in human plasma. Based on a mean radioactivity above 10%, the following major metabolites could be identified: diastereomers of M1, M2 (R-95913) and M5 (R-106583). The metabolites of prasugrel found in human plasma, urine and faeces were also detected in mouse, rat and dog; the only exception being M16, which was only identified in the mouse. M16 is M10 conjugated to glucuronic acid and M10 was found in all species. Furthermore, the extent of formation of a given metabolite varied significantly by species. Metabolite M1 was formed in large amounts in humans and was detected in animal plasma, but quantification was not conducted in animals due to co-eluting of the radioactive peaks. Metabolites M2, M5, M7 and M14 were also formed in larger amounts in humans as compared to the animal species.

In dogs, the hydrolysis of prasugrel led to a formation of essentially equal amounts of the four enantiomers of R-95913. All enantiomers of the active metabolite R-138727 were generated from R-95913. The R-125690 and R-125689 enantiomers accounted for approximately 50-64% of the R-138727 in dog plasma and >99% in rat plasma. The CHMP also inquired about levels in mice and rabbits. It was shown that all tested animal species were exposed to the most potent of enantiomers of R-138727, R-125690 and R-125689, at concentrations significantly higher than those observed in humans and thus, adequate margins of safety could be assured. Also, all animal species were exposed to the least potent of R-138727 enantiomers at concentrations higher than those in humans, with the exception of the rat as R-125687 and R-125688 concentrations could not be quantified.

Considering that isomers can have different or even antagonistic effects towards the same receptor system, these opposite effects might occur in species are capable of forming all four enantiomers of R-138727. Nevertheless, in the course of several studies of the antagonistic profile of the enantiomers using light transmission aggregometry, no evidence of agonistic activity was noted between R-125690 and R-125689 isomers of R-138727.

When administered at high doses (≥100 mg/kg) to rats, prasugrel induced CYP450 enzymes (CYP2B and CYP3A2) and phase II metabolizing enzymes UDP-glucuronosyltransferase and glutathione-S-transferase, however, based on the *in vitro* non clinical study with human hepatocytes, this induction is not observed in humans. Furthermore, the AUC for each measured metabolite decreased after multiple dosing compared with the values obtained after the first dose in mice at ≥100 mg/kg/day, in rats at 100 and 300 mg/kg/day, and in dogs at 20 mg/kg/day (after 20 weeks of dosing and beyond). However, the exposure data in dogs administered prasugrel at 20 mg/kg for one month were essentially unchanged. In the nine month study with dogs, the AUC data for two of the metabolites R-100932 and R-106583 decreased after 20 weeks of dosing, while the AUC values of the other metabolite, R-95913 were higher in dogs dosed with prasugrel at 20 mg/kg. Thus, the data show some auto-induction of prasugrel's metabolism at the 20-mg/kg dose in dogs. Since induction was not observed either *in vitro* or *in vivo* in humans and the non-clinical data suggest that induction of CYP3A4 due to administration of prasugrel is unlikely at clinically relevant plasma concentrations, this is not considered a major issue.

In mice, 90% of the dose was excreted during the first 24 hours post dosing mainly *via* the urinary elimination route. In rats and dogs, the majority of the radioactivity (>90%) was excreted within the first 72 hours of dosing in faeces presumably *via* bile. Approximately 20% of the dose was excreted via urine. Radioactivity related to prasugrel was also detected in milk of lactating rats at

concentrations up to approximately five times higher than the plasma level. However, the radioactivity from milk ($T_{½}$=9.5 h) was eliminated more rapidly than that from the plasma ($T_{½}$ approximately 24 h).

No pharmacokinetic drug interactions studies were conducted in animals and this was justified with the sufficient evaluation of pharmacokinetic drug interactions in a clinical setting.

Toxicology

The toxicological and toxicokinetic profile of prasugrel was investigated in a comprehensive programme, including studies on systemic toxicity after single and repeat dose administration, reproductive toxicity studies, genotoxicity studies as well as studies addressing specific issues, such as antigenicity, phototoxicity, toxicity of impurities, dermal and ocular irritation.

Prasugrel base was used during the major part of the toxicology program. However, a change was made to the hydrochloride salt of prasugrel later in development. Repeat dose studies comparing the base and the hydrochloride salt of prasugrel were conducted in mice with two week duration and rats and dogs with one month duration. A single dose comparison study was conducted in rats.

- Single dose toxicity

Single dose toxicity studies following the oral administration were conducted in rats and mice at doses up to 2000 mg/kg. Clinical observations in female rats given 2000 mg/kg included some non-specific signs of irregular respiration, reduced locomotor activity, ptosis, lacrimation, and staggering gait. In a comparison single dose rat study of prasugrel base *vs* prasugrel hydrochloride, no deaths occurred at doses of prasugrel base up to 2000 mg/kg, while 3 out of 5 males and 4 out of 5 females administered 2000 mg/kg prasugrel hydrochloride died. Systemic exposure to prasugrel metabolites in the prasugrel hydrochloride group was 1.2 to 3.5 times higher than that of the prasugrel base group and this difference is believed to account for the difference in mortality. In an escalating dose study in beagle dogs, platelet aggregation was inhibited, consistent with the pharmacological action of the compound. Emesis was observed after administration at doses ≥300 mg/kg, and serum ALP was increased following the 2000 mg/kg dose. Slight hepatocellular atrophy and ground glass appearance of hepatocellular cytoplasm were also observed in these dogs. Data from mice, rat and dog showed that prasugrel has low acute toxicity.

- Repeat dose toxicity (with toxicokinetics)

Repeat dose studies of up to three, six, and nine months in duration were conducted with prasugrel administered orally to mice, rats, and dogs. In most of these studies prasugrel base was used as the tested compound. Bridging studies comparing the toxicity of prasugrel base and prasugrel hydrochloride were conducted in each species.

Mortality, decreased body weight, and anaemia were observed in mice at repeated administration of a dose of 1000 mg/kg prasugrel. Anaemia was attributed to subclinical blood loss rather than to haematopoietic suppression, since an increase in the reticulocyte ratio was also observed, and there were no histologic effects on bone marrow. Liver was the primary target organ and increased liver weight and hypertrophy of centrilobular hepatocytes most likely due to induction of drug metabolising enzymes were observed. In the two week study, increased ALT and AST activity and the single cell necrosis indicated toxic effects on liver at a 2000 mg/kg dose of prasugrel, which was also lethal. The maximum tolerated dose (MTD) of prasugrel in mice was considered to be 300 mg/kg. Similar effects were observed in a fourteen day bridging study conducted in mice to compare the toxicity of prasugrel base and prasugrel hydrochloride. Some effects, e.g. the decreased erythrocytic parameters and liver histopathology findings, were more apparent in the prasugrel hydrochloride group.

No animals died during the studies in which rats were administered 0, 10, 30, 100, and 300 mg/kg of prasugrel orally for 3 months. Body weights decreased relative to control by 10% and 6% for males and females, respectively, in the 300 mg/kg group. Platelet counts increased in males given ≥100 mg/kg and in females given 300 mg/kg. Prothrombin times in males and activated partial thromboplastin times were prolonged in rats receiving ≥100 mg/kg. Evidence of enzyme induction included increased liver weight, hypertrophy, and acidophilic cytoplasm of hepatocytes, in male rats

given ≥100 mg/kg and female rats given 300 mg/kg. The enzyme induction effects and alterations in coagulation parameters were considered compensatory and pharmacologic in nature and thus not adverse.

Administration of similar doses of prasugrel orally for 6 months did not result in any deaths and similar blood effects were observed at higher doses. Clinical chemistry effects included decreased total cholesterol in males of the 300 mg/kg group, decreased triglycerides in males of the 100 mg/kg and slight decrease in potassium and chloride in females of the 300 mg/kg group. These changes were attributed to decreased food consumption. Increased total bilirubin, total protein and β-globulin and albumin were thought to be caused by the acceleration of protein synthesis in the liver accompanying induction of drug metabolizing enzymes. An increase in calcium was observed in both sexes given doses ≥100 mg/kg and was considered to be due to the increase in serum protein and the consequent increase in protein bound calcium. Liver weight increase was noted. Histopathological examination revealed hypertrophy of the hepatocytes and are consistent with the occurrence of enzyme induction. Other changes included decreased thymus weight in females of the 100 and 300 mg/kg groups, decreased prostate weight in the 100 and 300 mg/kg groups, and decreased uterine weight in the 300 mg/kg group. These were all slight changes without accompanying histopathological changes. The NOAEL of prasugrel in this study was 30 mg/kg.

Prasugrel base and prasugrel hydrochloride were administered daily for 28 days to rats to examine their differences in toxicity. Prasugrel hydrochloride was administered at dose levels of 0, 30, 100, and 300 mg/kg, and prasugrel base was administered at 300 mg/kg. Decreased body weight gain associated with decreased food consumption was recorded in females at 100 mg/kg and in males and females at 300 mg/kg with prasugrel hydrochloride. Administration of prasugrel hydrochloride was associated with a tendency toward decreased erythrocyte parameters in males and females at 300 mg/kg and an increase in reticulocyte percentage in females at 300 mg/kg. Platelet count, activated partial thromboplastin time, and fibrinogen were also increased. Anomalous levels of triglycerides, glucose, potassium, chloride, calcium, total protein, albumin, α2-globulin and ß-globulin were observed. These findings were comparable at 300 mg/kg between prasugrel hydrochloride and prasugrel base. The observed increase in liver weight, thought to be caused by an induction of drug metabolizing enzymes, was observed in males at 30 mg/kg and in males and females at 100 mg/kg and above in the prasugrel hydrochloride group and at 300 mg/kg in the prasugrel base group. Macroscopic examination revealed dark discoloration of the liver in males and females at 300 mg/kg for both compounds, and histopathological examination revealed hypertrophy of hepatocytes at each dose level for both compounds. The quantitative differences in exposure parameters and toxicological findings between prasugrel base and hydrochloride were discussed by the CHMP, especially in terms of the choice of the compound for the long term toxicological studies. It was, however, justified that the observed differences in animals treated with prasugrel hydrochloride or prasugrel base were not indicative of qualitative differences in toxicologic responses. The comparability of the pharmacokinetic and toxicity profiles between the base and the salt in bridging studies up to one month in duration in mice, rats, and dogs supported the appropriateness of using the salt for long term toxicology studies.

Beagle dogs were administered 0, 0.8, 4, or 20 mg/kg of prasugrel orally for 3 and 9 months. In animals receiving 4 mg/kg or more, hypertrophy of hepatocytes accompanied by the ground glass appearance of cytoplasm was observed. Animals receiving 20 mg/kg showed increased alkaline phosphatase activities and electron microscopic examination revealed a slight proliferation of the smooth endoplasmic reticulum in hepatocytes. These changes were considered to be due to activation of drug metabolism enzymes induced by administration of prasugrel. Decreased total cholesterol levels occurred in animals receiving 20 mg/kg.

An oral toxicity study to compare the toxicities of prasugrel base and prasugrel hydrochloride was conducted in which the compounds were administered orally once daily for 28 days. There were no compound related clinical signs or effects on body weight, food consumption, ophthalmology, electrocardiography, urinalysis, haematology, or gross pathology. Elevation of ALP activity occurred in males and females of the groups at 100 mg/kg prasugrel hydrochloride and 100 mg/kg prasugrel base. The increases in alkaline phosphatase levels occurred earlier and were more pronounced in the female dogs treated with 100 mg/kg prasugrel hydrochloride than in female dogs given 100 mg/kg

prasugrel base. The histopathological examination revealed lamellar inclusion bodies in the hepatocellular cytoplasm after administration of prasugrel hydrochloride. Hypertrophy of hepatocytes was observed with both compounds and was attributed to the induction of drug metabolizing enzymes. Slight hypertrophy of the thyroid follicular epithelia was observed in a male dog given 100 mg/kg prasugrel base. The changes observed in the thyroids were secondary to the accelerated metabolism of thyroid hormones due to elevated hepatic drug metabolizing enzymes.

The histopathological liver alterations and the serum hepatic enzymes changes were observed continuously throughout the repeat dose toxicity studies in mice, rats and dogs. The CHMP's concern regarding these findings, their relevance for prediction of human hepatotoxicity, especially considering that induction of CYPP450 is not observed in humans, and the overall potential hepatotoxicity of thienopyridines was appropriately addressed. Despite the lack of evidence for hepatotoxicity, hepatotoxicity is identified as a precautionary approach as a Potential Risk in the Risk Management Plan (RMP) and is subject to a range of surveillance activities.

- Genotoxicity

Prasugrel did not exhibit genotoxic properties when tested in a battery of standard *in vitro* (Ames and chromosome aberration) and *in vivo* (mouse micronucleus) assays. However, the CHMP requested the information concerning the purity of the tested batches and specifically, the impurity levels in the batches with regards to the genotoxicity tests. In response, the impurity level in the batches used for the pivotal toxicity studies were characterised based on an analysis of the actual amount of the impurities in the administered doses at the NOAEL. Sufficient levels have been achieved. In case of MFTP and PFTP, the level of impurities in the lots used for the *in vitro* genotoxicity studies are regarded as sufficient for qualification at the proposed specifications (0.20% and 0.15%, respectively). Although the proposed specification for OHTP (0.20%) cannot be deemed qualified by the *in vitro* genotoxicity studies, the margins of safety for the *in vivo* micronucleus study are significantly high to qualify the proposed specification for OHTP (>57 based on mg/m^2 and a prasugrel salt *vs* base exposure ratio of 3.5). Thus, OHTP, MFTP, and PFTP are considered qualified at the proposed specification.

- Carcinogenicity

Studies conducted over 24 months with mice at doses up to 300 mg/kg and rats with doses up to 100 mg/kg aimed at the assessment of the carcinogenic potential of prasugrel. When treated with prasugrel hydrochloride, mice developed adenomas of the liver, but not carcinomas. In view of the lack of genotoxicity, the increase in mice tumours was assumed to be caused by the adaptive enzyme induction response. The mice are prone to developing tumours under such circumstances and the mechanism is unlikely to be relevant for humans. Furthermore, hepatocellular hypertrophy, thought to be the result of microsomal enzyme induction, but no tumour induction was observed in the rat study. The increase in liver tumours in mice administered prasugrel is not considered to be a relevant human risk and this is adequately reflected in the proposed prescribing information.

- Reproduction Toxicity

Fertility, early embryonic development and peri- and postnatal toxicity were assessed in studies with rats and embryo-fœtal development in studies in rats and rabbits. In rats prasugrel did not exhibit toxicity on fertility and early embryonic development. In rabbits and rats prasugrel did not show signs of embryo-foetal toxicity. Prenatal and postnatal development, including maternal function in rats was not affected by exposure to prasugrel. The SPC adequately reflects these findings. The CHMP noted a reduction in mean adrenal gland, seminal vesicle/prostate gland, and combined epididymis weights at prasugrel doses of 300 mg/kg/day in the fertility rat study. There were no treatment-related histopathologic changes in these tissues in the 3- and 6-month rat studies and no effects in the dogs, except one early two week pilot study, in which atrophy of seminiferous epithelium in testes with slight-to-moderate nature was observed at high doses. This observation in dogs was comparable with the historic controls and did not appear in rats. Further evaluation of the data confirmed there were no effects on fertility, sperm count and sperm motility in rats. Overall, no reproductive risk could be concluded.

- Toxicokinetic data

Toxicokinetic data were collected from repeated dose studies in mice, rats and beagle dogs. Safety margins based on plasma drug exposures were determined for the active metabolite R-138727 and for R-106583 in the relevant studies. In addition, safety margins based on administered dose/body surface area have also been determined (please see *Pharmacokinetics*).

- Local tolerance

No study on local tolerance was performed. This is considered acceptable since prasugrel is administered orally. However, exposure of the skin or the eyes to prasugrel may occur during the manufacturing process. Two irritation tests were conducted in rabbits. In the hazard evaluation studies conducted in New Zealand white rabbits, prasugrel was a mild ocular irritant and its administration to the conjunctival sac of rabbits resulted in iritis and conjunctivitis, which resolved within 24 hours and seven days after the treatment, respectively. Prasugrel did not cause dermal irritation following a single application of 1000 mg/kg to the skin of rabbits.

- Other toxicity studies

Antigenicity

Prasugrel was tested for antigenicity in mice and guinea pig. Based on the results obtained from these tests, prasugrel is not expected to be antigenic.

Immunotoxicity

No specific tests for immunotoxicity were conducted and this was justified by the results of standard toxicity tests or based on pharmacologic properties of the compounds. It was argued that the available clinical safety data did not reveal any prasugrel related hypersensitivity reactions or suggest any increase in infection in the prasugrel *vs* clopidogrel treatment groups. There is no direct link between prasugrel and allergic reactions, but due to the fact that other thienopyridines have been associated with allergic reactions, these have been identified as potential risks in the RMP and are subject to a range of surveillance activities.

Phototoxicity

Distribution studies showed that prasugrel metabolites are distributed to the skin and eye ball in levels of 1/10 of the plasma concentration after single exposure, with some potential to accumulate after repeated dosing. The phototoxic potentials of R-138727 and R-106583 were evaluated *in vitro* examining the uptake of Neutral Red in the presence or absence of light using Balb/c 3T3 cells of mouse fibroblast cell line in the range of 290-700 nm. For R-138727, the Photo Irritation Factor (PIF) was below 2 (i.e. non-phototoxic). For R-106583, the PIF was not determined because the cell survival was >50%, with or without irradiation and indicated no remarkable cytotoxicity up to the maximum concentration of 1000 µg/mL. Nevertheless, R-138727 was determined as "probably phototoxic" in a second study employing the same dose range and experimental design, with a PIF of >2 (i.e., PIF 4.31). R-106583 was negative. According to the Note for guidance on phototoxicity testing (CPMP/SWP/398/01) it was not shown that prasugrel and/or its metabolites are not phototoxic and the CHMP raised a question on this issue. In response, it was shown that other non-clinical and clinical data indicate that evidence of the phototoxic potential of prasugrel is weak and of questionable clinical relevance. Nevertheless, phototoxicity was included as a potential risk in the RMP. The lack of photoallergy and photogenotoxicity is acceptable in light of the weak evidence of the phototoxic potential.

Studies on impurities

The potential toxicities of most of the prasugrel impurities were evaluated as part of the non clinical toxicology studies. All impurities above 0.15 % were qualified either by separate genotoxocity studies and a 14-day repeat dose study or toxicological studies. Based on the studies, the overall specifications for impurities CATP and diketone were considered justified from a toxicological perspective.

Ecotoxicity/environmental risk assessment

Environmental chemistry, fate and effects data were collected for prasugrel as recommended in the Guideline for environmental risk assessment of medicinal products for human use

(CHMP/SWP/4447/00). The Phase I estimate of maximum exposure to all prasugrel residue in surface water predicted an exposure above the 0.01 µg/L and thus, a complete risk assessment (Phase II Tier A) according to the current guideline has been conducted. No likely risk has been identified with regard to aquatic organisms in either ground water or surface water, neither for sediment dwelling organisms.

2.4 Clinical aspects

Introduction

This full application concerns centralised procedure in accordance with the Regulation (EC) No 726/2004, Article 3(2)(a). It is submitted in accordance with Article 8(3) in Directive 2001/83/EC for a new active substance. Conditional approval, an approval under exceptional circumstances or an accelerated review are not requested

Prasugrel is an inhibitor of platelet activation and aggregation through the irreversible binding of its active metabolite to the $P2Y_{12}$ class of ADP receptors on platelets. Since platelets participate in the initiation and/or evolution of thrombotic complications of atherosclerotic disease, inhibition of platelet function can result in the reduction of the rate of cardiovascular events such as death, myocardial infarction, or stroke.

The approved indication is:
EFIENT, co-administered with acetylsalicylic acid (ASA), is indicated for the prevention of atherothrombotic events in patients with acute coronary syndrome (i.e. unstable angina, non-ST segment elevation myocardial infarction [UA/NSTEMI] or ST segment elevation myocardial infarction [STEMI]) undergoing primary or delayed percutaneous coronary intervention (PCI).

Scientific advice for the product was requested from the CHMP in 2004. The given advice concerned, among others, the population included in the clinical development programme and the number of studies to be conducted, choice of a comparator and clinical endpoints in the clinical studies, use of aspirin as co-therapy or monitoring of safety of patients. It is claimed that the relevant scientific guidelines were followed.

There is no paediatric development programme. According to the European legislation valid at the time of submission, there was no need to submit a paediatric investigation plan before July 2008.

At the time of submission, the prasugrel clinical development program consisted of 46 completed placebo-controlled or active-comparator (clopidogrel) controlled studies. In the majority of studies, subjects were randomly assigned, in an open-label or blinded fashion, to treatment using either parallel or crossover designs. Across all studies, 8656 subjects received at least one dose of prasugrel.

Summary of the key studies in the prasugrel clinical development program.

Study Alias	Study Type	Subjects (N)	Overall Conclusions
H7T-EW-TAAA, H7T-EW-TAAE, H7T-EW-TAAJ	Phase 1 Dose Ranging (single dose, multiple dose regimens) +/- aspirin Doses from 5 mg - 60 mg; daily multiple doses of 5 - 15 mg for up to 21 days	Healthy TAAA (42) TAAE (45) TAAJ (68)	Higher, faster, and more consistent IPA versus 300-/75-mg LD/MD clopidogrel
H7T-EW-TAAD	Phase 1b Dose Ranging (multiple LD/MD regimens) 28-day duration	Stable atherosclerosis (101)	Higher, faster, and more consistent IPA versus300-/75-mg LD/MD clopidogrel
H7T-MC-TAAH	Phase 2 Dose Ranging Safety (multiple LD/MD regimens) 30-day duration	Elective and urgent PCI (905)	60-/10-mg LD/MD prasugrel showed comparable TIMI Major – Minor bleeding to 300-/75-mg LD/MD clopidogrel, trend towards decreased 30-day MACE
H7T-MC- TABR	Phase 1b Comparative PK/PD (60-/10-mg LD/MD prasugrel vs 600-/75-mg LD/MD clopidogrel regimens) 28-day duration	Stable CAD (110)	More rapid onset of higher and less variable IPA versus 600-/75-mg LD/MD clopidogrel
H7T-MC-TABL	Phase 2 Comparative PD (60-/10-mg LD/MD prasugrel vs 600-/150-mg LD/MD clopidogrel regimens). 30-day duration	Elective PCI (201)	More rapid onset of higher IPA versus 600-/150-mg LD/MD clopidogrel
H7T-MC-TAAL	Phase 3 Pivotal Study (60-/10-mg LD/MD prasugrel vs 300-/75-mg LD/MD clopidogrel regimens) with aspirin Maximum duration 15 months	PCI in ACS (13608)	Superior efficacy for 60-/10-mg LD/MD prasugrel vs 300-/75-mg LD/MD clopidogrel regimens with higher risk of bleeding

Abbreviations: ACS = acute coronary syndromes; CAD = coronary artery disease; IPA = inhibition of platelet aggregation; LD = loading dose; MACE = major adverse cardiovascular events; MD = maintenance dose; N = number randomly assigned to prasugrel and/or clopidogrel; PCI = percutaneous coronary intervention; PD = pharmacodynamic; PK = pharmacokinetic; PK/PD = pharmacokinetic/pharmacodynamic; TIMI = Thrombolysis In Myocardial Infarction. [IMO. Source Module 5.3.6]]

GCP

As claimed by the applicant, clinical trials were performed in accordance with GCP. A statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC was also provided. The assessment of the clinical data did not raise concerns about their compliance with GCP. No inspection was requested.

Pharmacokinetics

Prasugrel is administered as a racemic prodrug that is metabolized *in vivo* to the active moiety, R-138727, which irreversibly binds to the platelet $P2Y_{12}$ receptor and blocks activation and aggregation induced by the $P2Y_{12}$ agonist adenosine diphosphate (ADP). The R-138727 metabolite is formed very rapidly during first-pass metabolism.

The pharmacokinetics of prasugrel's active metabolite (R-138727) in healthy subjects was assessed in various clinical pharmacology studies by conventional non-compartmental methods and by population analysis. A meta-analysis of non-compartmental pharmacokinetics estimates from 16 phase 1 studies consolidated exposure estimates from 506 healthy male and female subjects and evaluated the effect of specific subject factors on exposure to the active metabolite.

Formulation development
Prasugrel development began with prasugrel base, which was used in the earlier studies in healthy subjects and subjects with stable atherosclerosis. Decision to switch to prasugrel hydrochloride was made after study TAAC showed that the C_{max} and AUC of prasugrel's inactive metabolites were greatly reduced when prasugrel base was given to healthy subjects whose gastric pH was >6 at the time of dosing. This was believed to be of a potential consideration for patients taking concomitant treatment with proton pump inhibitors (PPIs) or H_2-receptor antagonists, which also raise gastric pH. Because the solubility of prasugrel hydrochloride is higher than that of prasugrel base at higher pH, switching from the base to the hydrochloride salt might lessen the impact of elevated gastric pH in patients taking PPIs and H_2-receptor antagonists. Formulation strategy for the hydrochloride salt of prasugrel focused on developing an immediate-release tablet for oral administration. Initially, a 10-mg tablet was developed, which is to be used for both, the 60-mg loading dose (LD) and the daily 10-mg maintenance dose (MD). Later a 5-mg tablet was developed to provide increased dosing flexibility. The proposed commercial 10-mg tablet formulation was used in the pivotal, phase 3 study TAAL, and thus, no bioequivalence study was performed.

- Absorption

Prasugrel is rapidly absorbed after oral administration and is not detected in plasma. However, prasugrel's active metabolite (R-138727) appears in plasma rapidly after the oral dosing, reaching a peak concentration (C_{max}) in about 30 minutes and declining bi-phasically with a terminal half life of approximately 7.4 hours. The average C_{max} of active metabolite is 475 ng/ml after a 60-mg LD and 70 ng/mL during 10-mg MD. The time to reach the maximum plasma concentration (t_{max}) is at a median of 0.5 hours. It was estimated that approximately 79% of a prasugrel dose is absorbed. The between-subject and within-subject variability is 27.6% and 19.3%, respectively, for active metabolite AUC, and 30.1% and 38.1% respectively, for active metabolite C_{max}.

It was found that two 5-mg prasugrel hydrochloride tablets were bioequivalent with one 10-mg prasugrel hydrochloride tablet. During tablet manufacturing and storage, prasugrel hydrochloride tablets can convert to prasugrel base. The conversion from salt to base up to 70% has no impact on the extension and rate of the bioavailability of prasugrel at normal gastric pH, and furthermore, as study TACR confirmed, a 5 to 70% conversion of prasugrel hydrochloride tablets to prasugrel base did not affect the AUC or C_{max} of R-138727 in healthy subjects with normal gastric pH. There is a procedure in place with the aim of controlling the conversion and keeping it within this rate. The influence of food was assessed with a 25 mg and 15 mg dose of prasugrel. One of the effect of food on R-138727 AUC was the lower absorption rate, with C_{max} being 48.8% lower in the fed state, t_{max} delayed from 0.5 to 1.5 hours. Although an important pharmacokinetic parameter during maintenance dose is AUC, C_{max} is considered relevant in patients who receive a loading dose in order to achieve a more rapid onset of the pharmacological effect. Although the percutaneous coronary intervention (PCI) is usually performed in the fasted state, it is necessary to point out the importance of the administration of prasugrel loading dose in fasted state in the SPC. The CHMP thus proposed to revise the SPC wording to reflect that the onset of action of the loading dose may be most rapid in the fasted sate and this new wording was accepted.

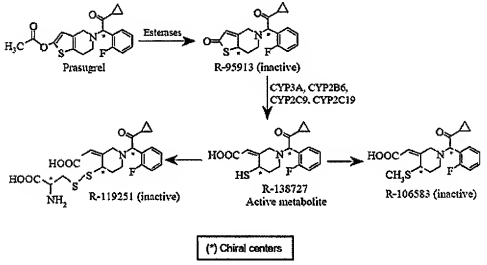
- Distribution

Estimates of apparent volume of distribution of R-138727 ranged between 40.3-66.4 l in healthy subjects and subjects with stable atherosclerosis. Prasugrel metabolites demonstrated limited penetration into red blood cells and the plasma-to-whole blood ratio was generally greater than one suggesting that radioactivity in the plasma was greater than that in an equivalent volume of blood cells. Because R-138727 is unstable in plasma, its binding to plasma proteins could not be determined. However, in a 4% human serum albumin solution in phosphate buffer at pH 7.4, R-138727 was 98% bound. For the inactive metabolites, the fraction bound to plasma proteins at various concentrations determined by ultracentrifugation, was 94.6% for R-95913, 95.1% for R-106583, and 76.4% for R-119251. Thus, the active metabolite is highly bound to protein and the measured concentration will depend on the protein content, which may be influenced by factors such as renal function, age and concomitant medication. However, only a minor fraction is unbound and this is not likely to change

significantly. Although the total concentration of the active metabolite might be lower, the effect of the drug may be similar patients with renal failure to that found in healthy persons.

- Elimination

Prasugrel is *in vivo* rapidly hydrolysed by esterases and the product of this hydrolysis, the pharmacologically inactive thiolactone R-95913, is metabolised to the active metabolite R-138727 mainly by cytochrome P450 CYP3A and CYP2B6, and, to a lesser extent, by CYP2C9 and CYP2C19. R-138727 is further metabolised to two inactive compounds by S-methylation or conjugation with cysteine (R-119251 and R-106583). Other prasugrel metabolites are formed by oxidation and/or conjugation and are not pharmacologically active. In case of the active metabolite R-138727, which is eliminated by S-methylation and conjugation with cystein, it is unclear which enzyme is involved in the elimination of the active metabolite. The CHMP was concerned about the clinical relevance of this issue and requested further clarifications. Based on the *in vitro* study, it would appear that thiopurine S-methyltransferase (TPMT) is not responsible for the S-methylation of R-138727 to R-106583 and that the S-methylation appeared mainly in human liver microsomes. Formation of R-106583 was inhibited by an inhibitor of thiol S-methyltransferase (TMT). The results thus suggest that TMT, and not TPMT, is responsible for R-106583 formation from prasugrel's active metabolite in human liver. However, possible inhibition of TMT by other drugs is unknown. It is considered beneficial that the rapid and efficient generation of the active metabolite of prasugrel results in its rapid appearance in plasma and consequently, in a rapid and extensive inhibition of platelet aggregation. Prasugrel exposure appears to be essentially unaffected by CYP inhibitors, inducers, and competitive inhibition by CYP substrates.



Simplified prasugrel metabolic pathway.

Approximately half of the active metabolite amount appearing in plasma is formed during absorption and/or during first-pass metabolism in liver, which explains the rapid appearance of active metabolite in plasma. Other prasugrel metabolites are formed by oxidation and/or conjugation and are not pharmacologically active.

Approximately 95% of a [^{14}C]prasugrel dose was recovered after oral administration. It was estimated that ca 68% of the prasugrel dose is excreted in urine and 27% in faeces in form of the inactive metabolites over a period of 10 days. Thus, urinary excretion is the major pathway for the elimination of prasugrel metabolites. The elimination half-life of R-138727 is about 7.4 hours. No R-138727 is detected in urine or faeces.

- Dose proportionality and time dependencies

Time dependency has not been specifically addressed. Several clinical studies support the evidence that exposure to prasugrel's active metabolite is dose-proportional. Furthermore, the comparison of $AUC_{(0-t)}$ and C_{max} values for the active metabolite with the dose shows a linear relationship with no discernable deviation from linearity over the entire dose range of 5 - 60 mg.

- Special populations

Impaired renal function

The effect of renal impairment on the disposition of prasugrel metabolites and platelet aggregation was investigated in three clinical studies (TAAO, TABW, and TACJ). Included were subjects with end stage renal disease, subjects with moderate renal impairment

Moderate Renal Impairment and End Stage Renal Disease (ESDR)

The $AUC_{(0\text{-}tlast)}$ and C_{max} values for the active metabolite R-138727 both averaged 38% lower in subjects with ESRD on dialysis across the dose range of 5- 60mg than in subjects with normal renal function. The lower active metabolite exposure in subjects with ESRD is generally consistent with an analysis across all three studies, TAAO, TABW and TACJ, in subjects with ESRD who received a 60-mg LD of prasugrel. Despite the differences in active metabolite exposure, platelet aggregation response to prasugrel is similar in ESRD and healthy subjects. Although subjects older than 65 typically have some degree of renal impairment, no differences in AUC or C_{max} of the active metabolite were observed in a clinical setting. Exposure to the active metabolite was comparable in subjects with moderate renal impairment (estimated creatinine clearance of 30-50 mL/min) and matched healthy controls; although median exposure to prasugrel's active metabolite was higher by approximately 22% in subjects with mild renal impairment than in subjects with normal renal function. The analyses of subjects with renal impairment in clinical pharmacology studies and in the substudy in phase 3 trial do not support the need for a dose adjustment for renal impairment. The CHMP, however, requested more information regarding the observed inconsistency in the pharmacokinetic parameters, especially as patients with end stage renal function seem to have lower levels of the active metabolite compared to healthy subjects. Plausible explanations for the comparable efficacy between ESRD patients and healthy adults were provided and these do not suggest that the dose adjustments are warranted. Nevertheless, the risk of bleeding episodes may be increased in patients with ESRD and the need for caution is reflected in the SPC.

Impaired hepatic function

Two Studies were performed in patients with mild to moderate hepatic function (Child-A and Child-B). Based on these results no dose adjustment in this population appears necessary, however, caution should be exercised in patients with moderate hepatic impairment. Clinical trials performed with prasugrel have not included patients with severe hepatic impairment (Child-C). As this population has a higher risk of bleeding, a contraindication in the SPC for patients with severe hepatic impairment (Child Pugh Class C) was included.

Gender

The pharmacokinetic meta-analysis of 16 clinical pharmacology studies detected no effect of gender on the exposure to prasugrel's active metabolite.

Race

The effect of ethnic origin was assessed in the pharmacokinetic meta-analysis of 16 clinical pharmacology studies. Most of the 437 subjects evaluated after a prasugrel LD and 284 subjects evaluated during prasugrel MD were Caucasian, although about 22% were Asian. Most Asian subjects in the meta-analysis originated from the three clinical pharmacology studies specifically designed to assess the influence of Asian ethnicity on prasugrel pharmacokinetics and pharmacodynamics. In each of these studies, Caucasian subjects served as the reference population.

Active metabolite exposure was similar in Chinese, Japanese, and Korean subjects after a 60-mg LD and during 10-mg and 5-mg MDs. However, the analysis showed that $AUC_{(0\text{-}tlast)}$ in Asians was 40% higher during MD and compared to Caucasians, the higher exposures in Asians produced higher inhibition of platelet aggregation (IPA) at most time points. Asians and Caucasians in the LD portion

of the pharmacokinetic meta-analysis had mean body weights of 65 kg and 77 kg, respectively, so the meta-analysis compared weight normalised $AUC_{(0\text{-}tlast)}$ and C_{max} values between Asians and Caucasians to assess the contribution of weight to exposure. After adjusting for body weight, the $AUC_{(0\text{-}tlast)}$ of the active metabolite was still approximately 19% higher in Chinese, Japanese, and Korean subjects compared to that of Caucasians, predominantly related to higher exposure in Asian subjects <60 kg. No dose adjustment is recommended based on ethnicity alone, but therapeutic experience with prasugrel is limited in Asian patients and therefore, prasugrel should be used with caution.

Weight

Analyses of several clinical studies in healthy subjects, subjects with stable atherosclerosis and subjects with acute coronary syndrome undergoing PCI consistently show that the AUC of the prasugrel active metabolite increases with a decreasing body weight. The relationship between the body weight and the active metabolite AUC was assessed using a conventional statistical approach that relied on univariate and multivariate analyses to quantify the magnitude of the body weight effect on active metabolite exposure. In healthy subjects, weight was one of the two covariates declared clinically significant in a multivariate analysis of these data, the other being Asian ethnicity. The univariate analysis supports consideration of dose adjustment at any weight threshold from ≥55 kg through 80 kg, while the multivariate analysis supports dose adjustment consideration at any weight threshold from ≥50 kg through 80 kg. In subjects with ACS undergoing PCI in Study TAAL, weight was one of the tree covariates declared significant during a multivariate analysis of these data, the other two being age and gender. The univariate analysis of the body weight effect supports consideration of dose adjustment for subjects <70 kg, but not for subjects ≥75 kg. The multivariate analysis of the body weight effect supports dose adjustment consideration for subjects <55 kg, but not for subjects ≥59 kg. The similarity in conclusions between the univariate and multivariate analyses clearly show that body weight is an important covariate. Further analyses of the risk for TIMI bleeding by different weight indicate that the odds ratio for bleeding with 10 mg prasugrel increases rapidly in the vicinity of 60 kg (and 75 years of age); supporting these values as cut-off choices for dose adjustment. A PK/PD model to assess the effect of the reduced dose (5 mg) in subjects < 60 kg or ≥75 years of age was developed and although the results are reassuring, clinical confirmation is needed (please see section Clinical Efficacy). Thus, the CHMP accepted the follow up measure to conduct a clinical study in subjects with stable CAD to compare the PK, PD, safety, and tolerability of prasugrel in subjects <60 kg to that of subjects ≥60 kg. Subjects will be treated with a maintenance dose of either prasugrel 5-mg, prasugrel 10-mg, or clopidogrel 75-mg. This study will exclude subjects ≥75 years. In addition, the SPC advises that the 10 mg maintenance dose is not recommended in subject weighing <60 kg.

Elderly

Age was one of 3 covariates declared statistically significant during a multivariate analysis of TAAL data as described above. When exposure was normalized by body weight, the 90%CI for the effect of age was below 1.25 for all age thresholds from 50 to 80 years old. Despite the lack of relationship between age and AUC in the multivariate analyses above, safety analyses of study TAAL revealed a strong relationship between bleeding risk and age, with a higher rate of bleeding in subjects ≥75 years old compared to those <75 years old. This prompted more extensive assessments of active metabolite exposure in the elderly.

The analysis focused on exploring the differences in exposure in patients approximately at and below the median age in the TAAL study compared to exposure in patients whose age was above specific thresholds up to 85 years. Based on this, a consideration of dose adjustment should be made at 70 years and more, with specific dose recommendations and the age thresholds associated with those recommendations depending on safety. Furthermore, an assumption was made about the anticipated clinical use of prasugrel where patients <60 kg would receive a 5-mg MD rather than a 10-mg MD, and patients ≥60 kg would then be considered for dose adjustment based on their actual age. In this approach a univariate analysis of age effect in subjects ≥60 kg is clinically more relevant and when this subgroup of patients ≥60 kg is assessed, the active metabolite AUC for patients ≥74 years is significantly larger than that for patients <74 years. Consistent with this difference, more than 60% of

patients ≥60 kg and ≥75 years old had concentrations above the median exposure in the TAAL study. This supports consideration of dose adjustment at any age threshold of 75 years or older, although specific dose recommendations and the age thresholds associated with those recommendations depend on safety.

In summary, age is a significant risk factor for bleeding. A cut-off level of 75 years based on a pharmacokinetic univariate analyses in subjects ≥ 60 kg is suggested.. This issue was addressed during the Scientific Advisory Group (SAG) meeting and in the oral explanation held at the CHMP meeting (please see section on Clinical Efficacy). Based on the CHMP discussions following the SAG meeting, Company written responses and oral explanation, the CHMP requested a strict SPC wording, which advises that the use of prasugrel in patients ≥ 75 years of age is generally not recommended. If use is deemed necessary based on careful individual benefit/risk evaluation by the prescribing physician, then following a 60 mg loading dose a reduced maintenance dose of 5 mg should be prescribed. An educational programme with regard to this topic is part of the conditions for the safe and effective use of the product (see sections 2.4 and 2.5). The results of the analysis conducted *via* a PK/PD model to evaluate the dose 5 mg in patients < 60 kg or ≥ 75 years of age need clinical confirmation and the Company is conducting such studies as part of the follow-up measures..

- Pharmacokinetic interaction studies

In vitro, prasugrel metabolites R-138727 and R-106583 did not inhibit the activities of cytochrome P450 CYP2D6, CYP2C9, CYP2C19, CYP1A2 and CYP3A4 hepatic isoforms up to 200 μM. The other major metabolite, the hydrolysis product R-95913, did not inhibit CYP1A2, but did inhibit CYP2D6, CYP2C9, CYP2C19 and CYP3A4. The projected maximum inhibition ranged from 2% for CYP2C9 to 21% for CYP2C19. None of these effects were deemed as a cause of a significant effect in the clearance of drugs metabolised by these enzymes. The effect of prasugrel on CYP1A2 and CYP3A4 was also assessed in primary cultures of human hepatocytes from four donors at various concentrations. No effect was observed on CYP1A2, but R-95913 showed a slight to moderate induction of CYP3A4 at a clinically relevant concentration. In order to further assess the clinical consequences of the signals detected from *in vitro* studies, a number of *in vivo* studies, including the assessment of pharmacokinetic interactions with aspirin, rantitidine, ketoconazole (CYP3A4/5 inhibitor), rifampicin (inducer of several CYP enzymes), atorvastatin, bupropion (a CYP2B6 substrate), warfarin, and heparin was conducted. An interaction study with digoxin was also conducted; aiming at the assessment of the effect of prasugrel on P-glycoprotein. Only a slight inhibitory effect of prasugrel on CYP2B6 (decreased hydroxibupropion exposure by around 20-30%) was observed. This effect is likely to be of clinical concern only if prasugrel is co-administered with drugs for which CYP2B6 is the only metabolic pathway and have a narrow therapeutic window. This concern of the CHMP is adequately expressed into the SPC of this medicinal product. Furthermore, inhibition or induction of CYP3A4 enzyme did not indicate any significant effect on prasugrel. Co-administration of prasugrel with digoxin at steady state did not significantly affect digoxin renal clearance and overall pharmacokinetics. Furthermore, prasugrel showed a lack of influence on the pharmacokinetics of S-warfarin, but caution should be exercised when prasugrel and warfarin are given together due to the potential increased risk of bleeding. Similarly, additional consideration is necessary during the co-administration of prasugrel with heparin as stated in SPC ("an increased risk of bleeding is possible when Efient is co-administered with heparin"). Daily co-administration of products elevating the gastric pH value, e.g. ranitidine or lansoprazole, did not change the metabolite's AUC and T_{max}, but decreased the C_{max} by 14% and 29%, respectively. Although in the maintenance therapy the C_{max} changes could be considered of less clinical relevance, in the loading dose when the intention is to achieve maximum inhibition of the platelet aggregation as quickly as possible, the C_{max} is considered a clinically relevant parameter. Therefore, a recommendation in the SPC that administration of the loading dose without concomitant administration with proton pump inhibitors may provide most rapid onset of action was included. In summary, the potential for pharmacokinetic interactions with prasugrel was adequately studied both *in vitro* and *in vivo*.

Pharmacodynamics

Platelets play a central role in the pathogenesis of atherothrombosis and in the formation of thrombi following coronary angioplasty, with and without stent implantation. Platelets initially adhere at sites of vascular injury, atherosclerotic plaque rupture, balloon angioplasty, and stenting. Platelet activation following these interactions results in the release of adenosine diphosphate (ADP), thromboxane A2, and other mediators. Released ADP promotes platelet activation *via* the G-protein linked $P2Y_1$ and $P2Y_{12}$ purinergic receptors leading to further platelet activation, aggregation, and other platelet functions, such as platelet shape change, secretion, and the development of pro-coagulant and pro-inflammatory activities. Activated platelets are recruited to sites of coronary plaque rupture and intra-arterial stenting, thereby forming aggregates that may lead to platelet-rich thrombi, vascular occlusion, tissue ischemia, and myocardial necrosis in what is collectively known as acute coronary syndromes.

Prasugrel is a thienopyridine ADP receptor antagonist that irreversibly inhibits platelet activation and aggregation mediated by the P2Y12 receptor. Prasugrel has a distinct chemical structure that permits efficient conversion to its active metabolite through rapid hydrolysis by carboxylesterases and then by multiple CYP450 enzymes.

- Mechanism of action

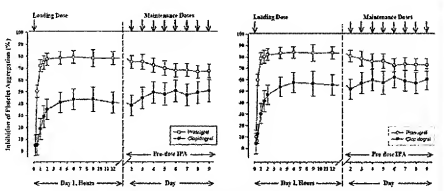
Prasugrel's pharmacological action is analogous to that described for other thienopyridines and results from covalent and irreversible binding of the active metabolite R-138727 to the $P2Y_{12}$ platelet ADP receptor. Once bound, a platelet is rendered ineffective for its remaining lifespan. After prasugrel administration is stopped, return to baseline platelet aggregation occurs only as new platelets are formed. The return to the baseline typically occurs at about 7 - 10 days after treatment is interrupted.

- Primary and Secondary pharmacology

Four initial studies assessing the safety, pharmacokinetic and pharmacodynamics of prasugrel in small numbers of healthy subjects allowed for the initial assessment of prasugrel activity, but did not analyze for prasugrel's active metabolite. Subsequently, four studies aimed at characterisation of the prasugrel hydrochloride salt and four initial clinical studies were conducted with prasugrel base to characterise pharmacokinetic, pharmacodynamics and tolerability in healthy subjects. Pharmacodynamics effects of thienopyridines on platelet function may be assessed by inducing platelet aggregation with various concentrations of ADP. Response to 20 µM ADP has been used as the primary pharmacodynamic parameter considering that it is a specific indicator of $P2Y_{12}$ function. Four clinical studies were conducted to evaluate the prasugrel-mediated inhibition of platelet aggregation and to characterise prasugrel's safety and tolerability, its pharmacokinetic and pharmacodynamic profile, effects on platelet function and bleeding time. In all tests, effective inhibition of platelet aggregation was observed with the onset of effect occurring within 1 hour of dosing. The effect continued through 48 hours post dosing. Platelet aggregation returned to normal levels at day 7. Reported adverse events included gastrointestinal disturbances, autonomic disturbances and general disorders, but none were serious.

A meta-analysis of pharmacodynamic data across the studies in healthy subjects and in subjects with stable atherosclerosis was conducted (see figure below) and the results indicate that within 30 minutes, the average inhibition of platelet aggregation (IPA) exceeds 50%. This time point is a key value, because it is the first assessed time point at which the IPA shows a statistically significant difference from baseline. Furthermore, within 1 hour, 97% of the subjects achieved an IPA above 20%, with the average IPA exceeding 70%. Over 89% of all subjects achieved at least 50% IPA by 1 hour, and over 90% of the maximum mean IPA is achieved by that time. One hour is a relevant time point because the average IPA across all subjects after a prasugrel 60-mg LD is nearly as high as the peak IPA eventually reached. By 4 hours, the average IPA is about 80%. More than 99% of the prasugrel subjects in the meta-analysis had an IPA above 20%, and about 90% of subjects achieved 90% of their individual maximum IPA by then. At each of these time points, a 300-mg clopidogrel LD showed a lower peak of IPA, fewer subjects achieved $\geq$20% IPA. The results in response to 5 µM ADP are similar. Following the administration of a single dose of prasugrel to healthy subjects not taking acetylsalicylic acid, platelet aggregation returned to normal levels by day 6 after a single administration of 30- or 75-mg dose of prasugrel base. After multiple doses of prasugrel to healthy

subjects taking acetylsalicylic acid, platelet aggregation returned to baseline levels in 5 days following discontinuation of MD at steady-state.



Abbreviations: CI = confidence interval; IPA = inhibition of platelet aggregation; LD = loading dose; MD = maintenance dose.
Note: Arrows (↓) indicate dosing.
Source: Meta-analyses of PK and PD, Figure APP2.7.2.12

Figure 2.7.2.10. Least squares mean (±95% CI) IPA to 20 µM (left panel) and 5 µM (right panel) after prasugrel 60-mg LD/10-mg MD and clopidogrel 300-mg LD/75-mg MD – PD Meta-analysis.

In summary, the results of the evaluation of the pharmacodynamic effects of prasugrel as an inhibitor of platelet aggregation were expressed as maximum platelet aggregation (MPA), which decreases with increasing pharmacodynamic response, and IPA, which is derived from the MPA determination and increases with increasing pharmacodynamic response. Clinical studies comparing the pharmacodynamic response of prasugrel with that of clopidogrel at the loading or loading/maintenance doses showed that the maximum mean IPA was achieved faster and was greater with prasugrel. Greater pharmacodynamic response for prasugrel is believed to be the result of the more rapid and more extensive formation of its active metabolite and has a less response variability compared to clopidogrel.

No relevant pharmacodynamic interactions were noticed when prasugrel is coadministered with unfractioned ranitidine, ketoconazole, atorvastatin, unfractionated heparin, digoxin and warfarin. There is an additive pharmacodynamic interaction between aspirin and prasugrel, in terms of suppression of platelet aggregation induced by collagen. The pivotal evidence of prasugrel in the claimed indication has been obtained as an add on therapy to low dose aspirin and thus, the potential safety risk of this interaction has been evaluated. In addition, the metabolic pathways for aspirin are separate from those for prasugrel and therefore no metabolic interaction would be expected. Co-administration of ketoconazole, a potent inhibitor of CYP3A4 and CYP3A5, with prasugrel did not significantly affect the exposure of the active metabolite of prasugrel, or the drug's effect on platelet inhibition. The possibility that the pharmacokinetics of prasugrel could be affected by inhibiting two or more pathways involved in prasugrel's metabolism was considered; however, only ticlopidine was listed as an acceptable CYP2B6 inhibitor. In addition, clopidogrel is the other drug that was clinically shown to be potent, mechanism-based inhibitor of CYP2B6. Because co-administering either of these drugs with prasugrel would not be considered in clinical practice, clinical evaluation of concomitant administration of prasugrel and of CYP2B6 (ticlopidine or clopidogrel) was not conducted. A clinical study aiming to assess the effect of 80 mg of prasugrel (single dose) on cardiac repolarisation was conducted including placebo and moxifloxacin as positive controls. The study design was in agreement with the ICH-E14 guideline. No effect of prasugrel on QT was observed above the threshold of regulatory concern (10 msec) at any time point. The positive control (moxifloxacine) showed a maximum QT prolongation effect within the expected range.

Clinical efficacy

The following studies have been conducted in order to support the use of prasugrel for the reduction of atherothrombotic events (CV death, nonfatal MI, or nonfatal stroke) in subjects with ACS:

Study Alias	Study Type	Subjects (N)	Primary Objective	Overall Conclusions
H7T-MC-TAAH	Phase 2 Dose Ranging Safety (multiple LD/MD regimens) Prasugrel (40-mg LD, 7.5-mg MD) Prasugrel (60-mg LD, 10-mg MD) Prasugrel (60-mg LD, 15-mg MD Clopidogrel (300-mg LD, 75-mg MD): All treatments were co-administered with aspirin. 30-day duration	Elective and urgent PCI (905)	1) Evaluate the safety of increasing doses of prasugrel by observing the rate of Non-CABG-associated significant bleeding (that is, Major plus Minor bleeding at 30 to 35 days after PCI). 2) Compare the safety of prasugrel to a standard regimen of clopidogrel (a 300-mg LD during PCI and 29 to 34 days of a 75-mg once daily MD) by observing the rate of Non- CABG- associated significant bleeding 30 to 35 days after PCI	60-/10-mg LD/MD prasugrel showed comparable TIMI Major + Minor bleeding to 300-/75-mg LD/MD clopidogrel, trend towards decreased 30-day MACE
H7T-MC-TABL	Phase 2 Comparative PD (60-/10-mg LD/MD prasugrel vs 600-/150-mg LD/MD clopidogrel regimens). 30-day duration	Elective PCI (201)	1) To compare the inhibition of platelet aggregation (IPA) with 20 µM ADP measured at 6 hours (±30 minutes) after prasugrel 60-mg LD versus clopidogrel 600-mg LD in subjects who did not receive a GP IIb/IIIa antagonist. 2) To compare the IPA with 20 µM ADP measured after 14±2 days of prasugrel 10-mg daily MD versus the IPA after 14±2 days of clopidogrel 150 mg daily MD in the "on-treatment population" who received PCI regardless of GPIIb/IIIa antagonist use (this included subjects receiving prasugrel and clopidogrel, in either order, during crossover)	60-/10-mg LD/MD prasugrel showed more rapid onset of higher IPA versus 600-/150-mg LD/MD clopidogrel
H7T-MC-TAAL	Phase 3 Pivotal Study (60-/10-mg LD/MD prasugrel vs 300-/75-mg LD/MD clopidogrel regimens) with aspirin Maximum duration 15 months	PCI in ACS (13608)	To demonstrate superiority of prasugrel co-administered with aspirin relative to clopidogrel co-administered with aspirin, as measured by a reduction in the composite endpoint of CV death, onfatal MI, or nonfatal stroke at a median follow up of at least 12 months.	Superior efficacy for 60-/10-mg LD/MD prasugrel vs 300-/75-mg LD/MD clopidogrel regimens with higher risk of bleeding

Abbreviations: ACS = acute coronary syndromes; CAD = coronary artery disease; IPA = inhibition of platelet aggregation; LD = loading dose; MACE = major adverse cardiovascular events; MD = maintenance dose; N = number randomly assigned to prasugrel and/or clopidogrel; PCI = percutaneous coronary intervention; PD = pharmacodynamic; PK = pharmacokinetic; PK/PD = pharmacokinetic/pharmacodynamic; TIMI = Thrombolysis in Myocardial Infarction.

- Dose response studies

Phase 3 dose selection was based primarily on 2 randomized clinical studies in subjects with stable atherosclerosis using the approved clopidogrel 300-/75-mg LD/MD regimen as the active comparator. The first study (TAAD) was a 28-day, phase 1b, dose-ranging pharmacokinetic/pharmacodynamic study in aspirin-treated subjects (N=101) comparing platelet inhibition using standard aggregometry. The second study (TAAH) was a 30-day, phase 2, dose-ranging safety study in aspirin-treated subjects (N=905) undergoing elective or urgent PCI (see below). Study TABL was conducted in parallel with the pivotal study TAAL to investigate the safety and pharmacodynamics of prasugrel against higher dose regimens of clopidogrel.

Study TAAD
This was a 28-day, phase 1b, dose-ranging pharmacokinetic/pharmacodynamic study in stable atherosclerosis patients (N=101) treated with aspirin (375 mg), in which the platelet inhibition was compared using the standard aggregometry. It is worth noting that the participants in this study are not entirely representative of those claimed in the indication of the current submission. In this study four different regimens (40 mg/5 mg, 40 mg/7,5 mg, 60 mg/10 mg and 60 mg/15 mg) were compared with the approved clopidogrel LD/MD regimen (300mg/75 mg). Overall, both the 40-mg and 60-mg prasugrel LDs resulted in more rapid onset with significantly greater IPA to 20 µM ADP from 2 to 6 hours after administration than the 300-mg LD of clopidogrel. Both the 10- and 15-mg prasugrel MDs achieved consistent and significantly greater IPA than the 75-mg clopidogrel MD; however, the 15-mg MD of prasugrel was associated with higher bleeding adverse events. In contrast, the prasugrel 5- and 7.5-mg MD groups were not consistently and statistically different in IPA from the clopidogrel 75-mg MD group.

Study TAAH
This was a double-blind, randomized, multicentre, dose-ranging trial of prasugrel compared with clopidogrel in subjects undergoing PCI. The primary endpoints evaluated the safety of increasing doses of prasugrel (a loading dose during PCI and once-daily maintenance dosing for 29 to 34 days) and compared prasugrel's safety with a standard regimen of clopidogrel (300-mg LD during PCI and 75-mg once-daily maintenance dose for 29 to 34 days) by observing the rate of non-CABG-associated significant bleeding (i.e. major and minor bleeding at 30 to 35 days after PCI).

The overall observed rate of all bleeding events was higher for subjects in the combined prasugrel group (29/650 subjects, 4.5%) compared with subjects in the clopidogrel group (9/254 subjects, 3.5%), but this difference was not statistically significant. With regard to the bleeding events, neither the differences among prasugrel dose groups (p=0.933), nor the differences between prasugrel and clopidogrel (p=0.590) were statistically significant. Thus, it was concluded that there was no statistically significant difference in the safety of increasing doses of prasugrel and no statistically significant difference between the safety of prasugrel and the standard clopidogrel regimen. The overall rate of non-CABG-associated significant bleeding was lower than anticipated and this resulted in reduced statistical power to assess the safety of prasugrel. A reduction of dose in the very elderly was recommended based on the pivotal study and is described later.

- Main studies

The pivotal phase 3 Study (H7T-MC-TAAL, further referred to as TAAL) was a global, multicentre, parallel-group, randomized, double-blind, double-dummy, active-controlled study. The primary objective of Study TAAL was to test the hypothesis that prasugrel is superior to clopidogrel in the treatment of subjects with ACS undergoing PCI, as measured by a reduction in the primary composite efficacy endpoint of CV death, nonfatal MI, or nonfatal stroke. This study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices (GCP) and the applicable laws and regulations of where the study was conducted. Study TAAL was evaluated based on several relevant guidelines:

- The CONSORT statement (The Lancet 2001;357:1191-94),
- Points to consider on the clinical investigation of new medicinal products for the treatment of unstable angina pectoris or non-Q-wave myocardial infarction, CPMP/EWP/570/98,
- Points to consider on application on one pivotal study (CPMP/EWP/2330/99),
- Points to consider on the clinical development of fibrinolytic medicinal products in the treatment of patients with ST segment elevation acute myocardial infarction (STEMI), CPMP/EWP/967/01.

In addition, scientific advice given by the CHMP in 2004 was considered when planning this pivotal study.

METHODS

Study Participants

Participants were to be of a legal age (and at least 18 years of age) and competent mental condition to provide written informed consent. For women of child-bearing potential, only those tested negative for pregnancy between ACS presentation and enrolment and agreed to use a reliable method of birth control during the study were included. Subjects with ACS (subjects with unstable angina and non-ST-segment elevation myocardial infarction [UA/NSTEMI] with TIMI risk score ≥3 or ST-segment elevation myocardial infarction [STEMI]) who are to undergo PCI were eligible to enter the study. The main inclusion criteria were:

- Moderate- to high-risk UA was defined as a history of chest discomfort or ischemic symptoms of ≥10 minutes duration at rest ≤72 hours prior to randomization, with persistent or transient ST-segment deviation ≥1 mm in one or more ECG leads without elevation of CK-MB or troponin T or I but with a TIMI risk score ≥3.
- Moderate- to high-risk NSTEMI was defined as a history of chest discomfort or ischemic symptoms of ≥10 min duration at rest ≤72 hours prior to randomization with no evidence of persistent ST-segment elevation. Subjects must also have CK-MB or troponin T or I greater than the upper limit of normal (ULN) and a TIMI risk score ≥3.
- ST-segment elevation myocardial infarction (STEMI) is defined as a history of chest discomfort or ischemic symptoms of >20 minutes duration at rest ≤14 days prior to randomization with one of the following present on at least one ECG prior to randomization: a) ST-segment elevation ≥1 mm in two or more contiguous ECG leads. b) New or presumably new left bundle branch block (LBBB). c) ST-segment depression ≥1 mm in two anterior precordial leads (V1 through V4) with clinical history and evidence suggestive of true posterior infarction.

Subjects with STEMI could be enrolled within 12 hours of symptom onset if primary PCI was planned or within 14 days if delayed PCI was planned following initial pharmacotherapy for STEMI.

Exclusion criteria were extensive. In general, these excluded subjects at increased risk of bleeding (for example, anaemia, thrombocytopenia, intracranial pathology, severe hepatic dysfunction, oral anticoagulants, chronic non-steroidal anti-inflammatory drug (NSAID) use, or use of any thienopyridine within 5 days of the main treatment), patients with refractory ventricular arrhythmia, class IV congestive heart. The inclusion/exclusion criteria are considered acceptable. Diagnosis and short-term risk stratification is based on the combination of ischaemic symptoms, ECG changes, biomarkers in some cases and risk score results. The recommendations of the European Society of Cardiology guidelines (2007) were followed.

Treatments

This study involved a comparison of prasugrel (60-mg LD, 10-mg MD) and clopidogrel (300-mg LD, 75-mg MD). Both treatments were administered orally as a one-time LD followed by a once daily MD. The loading and maintenance doses of prasugrel for this Phase 3 PCI study were selected on the basis of non-clinical thrombosis models, non-clinical toxicology studies, dose-escalation studies in healthy subjects, a dose-ranging study versus clopidogrel in subjects with stable coronary artery disease (Study TAAD), and a dose-ranging study versus clopidogrel in subjects undergoing elective or urgent PCI (Study TAAH). Owing to the link observed between thrombosis complications following PCI and poor antiplatelet response to clopidogrel, recommendations for the use of doses higher than the standard in PCI have been reported. There is evidence of some increase in the speed of onset and the level of platelet inhibition with both 600 mg and 900 mg of clopidogrel LDs. Still, these assumptions are based on limited data and require further sound confirmation. Thus, the use of the standard 300 mg LD (administration as soon as possible) and 75 mg/day MD of clopidogrel is acceptable.

Objectives

Primary objective: To test the hypothesis that prasugrel (co-administered with aspirin) was superior to clopidogrel (co-administered with aspirin) in the treatment of subjects with acute coronary syndromes who were to undergo percutaneous coronary intervention, as measured by a reduction in the composite endpoint of cardiovascular (CV) death, nonfatal myocardial infarction (MI), or nonfatal stroke at a median follow-up of at least 12 months.

The secondary efficacy objectives were to compare both treatments with respect to the:

- risk of cardiovascular (CV) death, nonfatal myocardial infarction (MI), or nonfatal stroke through 90 days.
- risk of CV death, nonfatal MI, or nonfatal stroke through 30 days.
- risk of CV death, nonfatal MI, or urgent target vessel revascularization through 90 days.
- risk of CV death, nonfatal MI, or urgent target vessel revascularization through 30 days.
- risk of all-cause death, nonfatal MI, or nonfatal stroke at study end.
- risk of CV death, nonfatal MI, nonfatal stroke, or rehospitalisation for cardiac ischemic events at study end.
- risk of definite or probable stent thrombosis per Academic Research Consortium (ARC) definition at study end.

The safety objectives of this study were to compare prasugrel with clopidogrel with respect to the:

- risk of non-coronary artery bypass graft (Non-CABG) Thrombolysis in Myocardial Infarction Study Group (TIMI) Major bleeding in subjects receiving prasugrel or clopidogrel.
- risk of Life-Threatening bleeding (a subset of Non-CABG-related TIMI Major bleeding) in subjects receiving prasugrel or clopidogrel.
- risk of Non-CABG-related TIMI Minor bleeding in subjects receiving prasugrel or clopidogrel.
- The overall safety and tolerability of prasugrel administration based on clinical findings, laboratory values, and the occurrence of treatment-emergent adverse events.

Outcomes/endpoints

The primary efficacy measure was time to first event of a composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke at study end. Cardiovascular Death (CV Death) was defined as death due to documented cardiovascular cause. Additionally, death not clearly attributable to non-cardiovascular causes was considered CV death. The definition of myocardial infarction (MI) was adapted from the standard American College of Cardiology (ACC) definition and was dependent on the clinical timing of the event in relation to presenting syndrome and cardiovascular procedures. A peri-procedural event must be distinct from the index event. If an ischemic biomarker was elevated at the onset of the suspected event, there must be demonstration of a falling biomarker level prior to the onset of the suspected event, and the subsequent peak must be greater than 1.5 times the value prior to the onset of the event. The biomarker levels required for the diagnosis of MI were dependent on relationship to cardiac procedures:

- If the suspected event was within 48 hours of a PCI, the CK-MB value (on at least two samples) must be >3x ULN or >5xULN on the last available sample provided it was obtained ≥12 hours after the PCI; no symptoms were required.
- If the suspected event was within 48 hours of a CABG, the CK-MB value (on a single measure) must be >10xULN; no symptoms were required.
- If the suspected event was not within 48 hours of a PCI or CABG, the diagnostic criteria were met if the subject had CK-MB or cardiac troponin >ULN and the presence of either chest pain ≥20 minutes in duration or ST-segment deviation ≥1 mm on the ECG.

In any clinical circumstance, the appearance of new Q-waves on the ECG distinct from a prior event or pathologic evidence (such as autopsy) showing a new MI felt to be distinct from a prior event were considered appropriate evidence for MI, as would ST-segment elevation lasting for at least 20 minutes and accompanied by ischemic chest pain or haemodynamic decompensation. Nonfatal stroke was defined as the rapid onset of new, persistent neurologic deficit lasting more than 24 hours. In the case of clinical diagnosis of nonfatal stroke, computed tomography (CT) or magnetic resonance imaging (MRI) scan imaging was strongly recommended. CT or MRI scans were considered by the Clinical Events Committee (CEC) to support the clinical impression. Supplemental information from head CT or MRI scans determined if there was a demonstrable lesion compatible with an acute nonfatal stroke. Furthermore, nonfatal stroke was classified as either ischemic or hemorrhagic based on imaging data, if available, or uncertain cause if imaging data were not available.

Secondary efficacy endpoints included:

- CV death, nonfatal MI, or nonfatal stroke through 30 days and 90 days post randomization.
- CV death, nonfatal MI, or UTVR through 30 days and 90 days post randomization. UTVR required both of the following: PCI or CABG, for recurrent ischemia that, in the investigator's opinion, could not be delayed for more than 24 hours and was defined by the investigator as a

non-elective procedure (urgent), and revascularization, either with CABG or PCI, had to include one or more vessel(s) dilated at the initial (qualifying) procedure.
- All cause death, nonfatal MI, or nonfatal stroke.
- CV death, nonfatal MI, nonfatal stroke, or re-hospitalization for cardiac ischaemic events.
- Definite or probable (Academic Research Consortium (ARC) definition) stent thrombosis.

Safety endpoints were set as follows: Non-CABG-related TIMI Major bleeding, Non-CABG-related TIMI Life-Threatening bleeding, and Non-CABG-related TIMI Minor bleeding.
Non-CABG-related TIMI Major bleeding was any intracranial haemorrhage (ICH) OR any clinically overt bleeding including bleeding evident on imaging studies) associated with a fall in haemoglobin (Hgb) of ≥5 gm/dL from baseline. Non-CABG-related TIMI Life-Threatening bleeding was any Non-CABG-related TIMI Major bleeding that was fatal, led to hypotension that required treatment with intravenous inotropic agents, or required surgical intervention for ongoing bleeding, or necessitated the transfusion of 4 or more units of blood over a 48-hour period, or any symptomatic ICH. Non-CABG-related TIMI Minor bleeding was any clinically overt bleeding associated with a fall in Hgb of >3 gm/dL but <5 gm/dL from baseline.

Sample size
Study TAAL was intended to continue until an estimated 875 subjects with UA/NSTEMI reached one of the events described in the triple composite endpoint (CV death, nonfatal MI, or nonfatal stroke) and a median duration of therapy of at least 12 months and a minimum follow-up of 6 months was achieved. A power calculation to assess superiority was performed, assuming a hazard ratio of 0.80 and based on a two-sided log-rank test using a two-sided significance level of 0.05. In view of an anticipated lack of proportionality of hazard, the Gehan-Wilcoxon test was used in the primary analysis. The statistical power of the Gehan-Wilcoxon test depends on the direction and size of the deviation from proportional hazards. It is expected, however, that the power of the Gehan-Wilcoxon test is approximately 90% if the non-proportionality is not severe. A total of around 13,000 subjects with ACS were to be enrolled, so that 9500 subjects with UA/NSTEMI would be enrolled. The number of subjects with STEMI was to be capped at 3500 subjects, which was deemed to be adequate to assess the consistency of treatment effect and safety within the STEMI population.

Randomisation
Subjects were randomized via an interactive voice response system (IVRS) to either prasugrel or clopidogrel in a 1:1 ratio. Randomization was carried out at the site level and stratified by clinical presentation. Subjects were randomized only after diagnostic angiography confirmed anatomy suitable for PCI, except for patients presenting with STEMI with onset of symptoms < 12 hours. Overall, the randomization procedure was assessed as successfully implemented. For a few patients (1.7%) the randomization was based on incorrect strata and the site monitoring identified a small number of patients (≤ 1.3%), who were given the wrong kit/drug. The impact of these protocol violations on the study results is believed to be negligible.

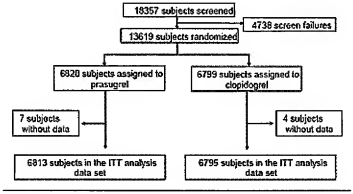
Blinding (masking)
Participants, the sponsors, and all study site personnel were blinded to study drug. Selected clinical study personnel not associated with the study or its operations were granted access to randomization table and treatment assignments. These personnel were limited to those who prepared unblinded summaries and analyses for the periodic safety reviews by the Data Safety Monitoring Board (DSMB) and/or regulatory agencies.

Statistical methods
All efficacy analyses were performed using an intent-to-treat dataset, consisting of all randomized subjects. The safety analyses were carried out using the treated data set that includes all subjects who received at least one dose of study drug, either a loading dose or maintenance dose. Primary efficacy analyses were conducted on endpoints adjudicated by an independent CEC, including CV death, myocardial infarction, stroke, urgent target vessel revascularization, and stent thrombosis. The comparison of the primary endpoint was evaluated using the Gehan-Wilcoxon test from a time-to-first event analysis at a two-sided alpha level of 0.05. Subjects experiencing multiple occurrences of an endpoint were censored at the time of first occurrence. All other key efficacy and safety analyses were

conducted using the log-rank test from a time-to-first event analysis. All efficacy and safety analyses were carried out first for subjects with UA/NSTEMI, followed by analysis of the same endpoint in the All ACS population (UA/NSTEMI plus STEMI subjects) if superiority of prasugrel was demonstrated in the UA/NSTEMI population. The primary outcome was also analyzed in the STEMI population. Analyses including all subjects with ACS included clinical presentation UA/NSTEMI *vs* STEMI as a stratification factor in the time-to-first event analyses. For the secondary analyses no formal adjustment of the grade of statistical significance is considered necessary, due to the presence of a predefined hierarchical strategy. The statistical analysis plan was well prepared and follows the recommendations in the CHMP Guideline CPMP/EWP/2330/99 (Points to Consider on multiplicity issues and on application with one pivotal study).

Results

Participant flow in study TAAL



There were 7 subjects randomly assigned to prasugrel, and 4 subjects randomly assigned to clopidogrel without data available for inclusion in the final analysis dataset due to an incomplete informed consent document. The most frequent reason for screen failure was that subjects did not meet the inclusion criteria. The majority (98.9%) of subjects completed the study and the number of patients withdrawing consent or considered lost to follow-up was similar between treatment groups.

Recruitment

The enrolment period for Study TAAL was 5 November 2004 to 14 January 2007. The last subject visit occurred on 22 July 2007. The geographic variation, which is likely to depict the future use of the drug, is shown below.

Region of Enrollment n (%)a	Prasugrel, n=6813	Clopdogrel, n=6795
North America	2164 (31.8)	2146 (31.6)
United States	2039 (29.9)	2020 (29.7)
Europe	3436 (50.4)	3439 (50.6)
Eastern Europe	1657 (24.3)	1665 (24.5)
Western Europe	1779 (26.1)	1774 (26.1)
South America	270 (3.96)	264 (3.89)
Rest of World	943 (13.8)	946 (13.92)

Conduct of the study

Study TAAL was conducted in 725 centres in 30 countries around the world. The following changes were made in the conduct of the study after the start of the study: The definition of nonfatal peri-procedural myocardial infarction (PPMI) within 48 hours after percutaneous coronary intervention was modified (protocol amendment A). The modified definition maintains the original definition and extends the definition of PPMI to include a CK-MB >5xULN on one sample if it is the last available sample and was drawn ≥12 hours after PCI. This change affected only the CEC adjudication of PPMI

within 48 hours of PCI. The criteria for investigator-identified nonfatal clinical MI that were also adjudicated by the CEC remained unchanged..
Overall, changes made during study conduct were not considered major.

Baseline data

Summary of baseline characteristics for subjects in study TAAL is presented below.

Characteristic	Prasugrel	Clopidogrel
Clinical Presentation n (%)	**N=6813**	**N=6795**
UA/NSTEMI	5044 (74.0)	5030 (74.0)
STEMI	1769 (25.9)	1765 (26.0)
STEMI ≤12 hours [a]	1203 (17.7)	1235 (18.2)
STEMI >12 hours [a]	564 (8.3)	530 (7.80)
Age (Years)	**N=6813**	**N=6795**
Overall Mean (SD)	60.9/11.2	60.9/11.4
≥75 Years n (%)[a]	901/13.2	908/13.4
Sex n (%a)	**N=6813**	**N=6795**
Female	1705 (25.0)	1818 (26.8)
Region of Enrollment n (%)a	**N=6813**	**N=6795**
North America	2164 (31.8)	2146 (31.6)
United States	2039 (29.9)	2020 (29.7)
Europe	3436 (50.4)	3439 (50.6)
Eastern Europe	1657 (24.3)	1665 (24.5)
Western Europe	1779 (26.1)	1774 (26.1)
South America	270 (3.96)	264 (3.89)
Rest of World	943 (13.8)	946 (13.92)
Body Weight (kg)	**N=6722**	**N=6715**
Mean (SD)	83.6 (16.8)	83.2 (16.9)
Creatinine Clearance	**N=6699**	**N=6681**
<60 ml/min	717 (10.7)	774 (11.6)
Index Procedure(s)	**N=6813**	**N=6795**
PCI	6715 (98.6)	6698 (98.6)
Multivessel PCI	967 (14.7)	946 (14.4)
CABG	25 (0.37)	23 (0.34)
Any Stent	6018 (95.7)	6004 (96.1)
Bare Metal Stent	3190 (51.0)	3185 (51.0)
Drug Eluting Stent	2860 (45.5)	2872 (46.0)
GPIIb/IIIa inhibitor use	3670 (53.9)	3733 (55.0)
Medical History n (%)[a]	**N=6813**	**N=6795**
Diabetes Mellitus	1576 (23.1)	1570 (23.1)
Hypertension	4370 (64.1)	4371 (64.3)
Hypercholesterolemia	3790 (55.6)	3790 (55.8)
Prior MI	1226 (18.0)	1208 (17.8)
Prior PCI	904 (13.3)	926 (13.6)
Prior CABG	541 (7.94)	497 (7.3)
Prior TIA	94 (1.38)	117 (1.7)
Prior Stroke	181 (2.66)	160 (2.35)

Abbreviations: CABG = coronary artery bypass graft; MI = myocardial infarction; N = number of subjects randomly assigned; n = number of subjects in sub-category; NSTEMI = non-ST segment elevation myocardial infarction; PCI = percutaneous coronary intervention; SD = standard deviation; STEMI = ST-segment elevation myocardial infarction; TIA = transient ischemic attack; UA = unstable angina.

[a] % is percent (rounded to nearest whole number) of number of subjects with non-missing values for category.

The majority of subjects were male and Caucasian. The mean age was 61 years and the mean weight was 83 kg. The subject characteristics were well balanced across the treatment groups; in UA/STEMI, STEMI, and all ACS populations. Exceptions of statistically significant differences were age and

diabetic treatment in the STEMI population, gender in the All ACS population, and the use of angiotensin-converting enzyme inhibitors (ACEI) in the UA/NSTEMI and the All ACS populations. The magnitude of the imbalances was small and these imbalances are not believed to affect the outcome of the study. It is of note that TIMI Risk Index score at baseline was identical in the all ACS population in the two treatment arms. There is a small difference - albeit statistically insignificant - in number of patients with a history of prior stroke between the two treatment arms.

Numbers analysed

The ITT population is defined as all randomized subjects except where otherwise specified. The Safety population is formed by all randomised patients who received at least one dose of the medication and had at least one contact with the investigator afterwards. Overall compliance with taking study drug was high (approximately 96%).

Outcomes and estimation

Study TAAL demonstrated that treatment with prasugrel, as compared with clopidogrel at the standard, approved dose, resulted in a statistically significant reduction in the rate of the primary efficacy endpoint (the composite of CV death, nonfatal MI, or nonfatal stroke at a median of 14.5 months follow-up). In addition, there was a statistically significant reduction in all pre-specified secondary efficacy endpoints. This was shown across the full spectrum of ACS with planned PCI. The primary and secondary efficacy endpoints were analyzed first in subjects with unstable angina and non-ST-segment elevation myocardial infarction (UA/NSTEMI), followed by an analysis of the same endpoints in all subjects in the All ACS population (UA/NSTEMI and ST-segment elevation myocardial infarction (STEMI)). The data were then also analysed in patients presenting with STEMI. For subjects presenting with UA/NSTEMI, the number and percentage of subjects reaching the primary composite endpoint were 469/5044 (9.30%) and 565/5030 (11.23%) for those randomized to prasugrel or clopidogrel, respectively, (HR=0.820 (95% CI 0.726-0.927)). For subjects presenting with STEMI, the number and percentage of subjects reaching the primary composite endpoint were 174/1769 (9.84%) and 216/1765 (12.24%) for those randomized to prasugrel or clopidogrel, respectively, (HR=0.793 (95% CI 0.649-0.968)). In the all ACS population, the number and percentage of subjects reaching the primary composite endpoint were 643/6813 (9.44%) and 781/6795 (11.49%) for those randomized to prasugrel or clopidogrel, respectively, (HR=0.812 (95% CI 0.732-0.902)). Thus, on average a relative risk reduction of approximately 20% was achieved. An absolute risk reduction of approximately 2% was observed.

Considering the individual components of the main endpoints significant reductions in the prasugrel group in the rates of ischemic events were observed. This differences were largely related to reduction in nonfatal MI (6.97% P *vs* 9.12% C, HR 0.757, p<.001) and all MI (7.12% P vs. 9.32% C, HR 0.757, p<.001). Positive results were evident within the first 24 hours following PCI, thus data seem to demonstrate a reduction in the early ischemic events such as peri-procedural MI. The risks of nonprocedural clinical MI were significantly reduced in the prasugrel group, as was the risk of new ST-elevation MI. However, this effect was not associated with a difference in the incidence of all cause death or CV death between treatment groups. It is of note that the higher level of platelet inhibition achieved relatively fast with the LD of prasugrel, leads to a reduction of the risk of thrombotic complications in the acute phase. Statistically significant differences in favour of prasugrel were also detected for all the planned secondary efficacy endpoints (see Methods, Objectives on page 35).

Study TAAL Primary Efficacy Endpoint and Components at Study End				
Event	**Prasugrel**	**Clopidogrel**	**Hazard Ratio**	**p-Value[c]**
	n (%)[a]	**n (%)[a]**	**(95% CI)[b]**	
UA/NSTEMI	**N=5044**	**N=5030**		
Primary End Point CV Death, Nonfatal MI, or Nonfatal Stroke	469 (9.30)	565 (11.23)	0.820 (0.726,0.927)	0.002
CV Death	90 (1.78)	92 (1.83)	0.979 (0.732,1.309)	0.885
Nonfatal MI	357 (7.08)	464 (9.22)	0.761 (0.663,0.873)	<0.001
Nonfatal Stroke	40 (0.79)	41 (0.82)	0.979 (0.633,1.513)	0.922
All Cause Death	130 (2.58)	121 (2.41)	1.076 (0.840,1.378)	0.563
All MI	366 (7.26)	476 (9.46)	0.760 (0.663,0.871)	<0.001
All Stroke	49 (0.97)	46 (0.91)	1.068 (0.714,1.597)	0.748
STEMI	**N=1769**	**N=1765**		
Primary End Point CV Death, Nonfatal MI, or Nonfatal Stroke	174 (9.84)	216 (12.24)	0.793 (0.649,0.968)	0.019
CV Death	43 (2.43)	58 (3.29)	0.738 (0.497,1.094)	0.129
Nonfatal MI	118 (6.67)	156 (8.84)	0.746 (0.588,0.948)	0.016
Nonfatal Stroke	21 (1.19)	19 (1.08)	1.097 (0.590,2.040)	0.770
All Cause Death	58 (3.28)	76 (4.31)	0.759 (0.539,1.068)	0.113
All MI	119 (6.73)	157 (8.90)	0.748 (0.589,0.949)	0.016
All Stroke	26 (1.47)	25 (1.42)	1.032 (0.596,1.787)	0.911
All ACS	**N=6813**	**N=6795**		
Primary End Point CV Death, Nonfatal MI, or Nonfatal Stroke	643 (9.44)	781 (11.49)	0.812 (0.732,0.902)	<.001
CV Death	133 (1.95)	150 (2.21)	0.886 (0.701,1.118)	0.307
Nonfatal MI	475 (6.97)	620 (9.12)	0.757 (0.672,0.853)	<0.001
Nonfatal Stroke	61 (0.90)	60 (0.88)	1.016 (0.712,1.451)	0.930
All Cause Death	188 (2.76)	197 (2.90)	0.953 (0.781,1.164)	0.639
All MI	485 (7.12)	633 (9.32)	0.757 (0.673,0.852)	<0.001
All Stroke	75 (1.10)	71 (1.04)	1.055 (0.763,1.460)	0.745

Abbreviations: ACS = acute coronary syndromes; CI = confidence interval; CV = cardiovascular; MI = myocardial infarction; N = number of randomly assigned subjects; n = number of subjects in sub-category; NSTEMI = non-ST-elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction; UA = unstable angina

[a]Percentage of randomly assigned subjects reaching the primary endpoint.

[b]Hazard ratio and a 95% CI used as an estimate of overall relative risk, prasugrel versus clopidogrel, over the course of the study.

[c]Two-sided p-values are based on Gehan–Wilcoxon test comparing event free survival distributions of prasugrel and clopidogrel for the composite primary endpoint. The individual components of the endpoints were tested using log-rank test. Clinical presentation, UA/NSTEMI versus STEMI, was used as a stratification factor in analysis involving All ACS subjects.

Efficacy was preserved across major pre-specified subgroups: sex, age (older or younger than 65 years), history of diabetes, type of stent employed, use of glycoprotein inhibitors, mono- or poly antithrombin use, dose of aspirin, renal function, geographical region. The treatment benefit was durable. The incidence of primary and secondary composite endpoints was statistically significantly lower in subjects treated with prasugrel compared to clopidogrel in the acute phase (within 3 days), the subacute phase (within 30 days), and in the long-term phase.

Secondary Outcome Events in Study TAAL – All ACS population				
Outcome Events	prasugrel + ASA (N=6813) %	Clopidogrel +ASA (N=6795) %	Hazard Ratio (95% CI)	p-value
CV death, nonfatal MI or nonfatal stroke through 90 days	6.8	8.4	0.797 (0.705,0.901)	<0.001
CV death, nonfatal MI or nonfatal stroke through 30 days	5.7	7.4	0.767 (0.672,0.876)	<0.001
CV Death, Nonfatal MI or urgent target vessel revascularisation (UTVR) through 90 days	6.9	8.7	0.794 (0.703,0.896)	<0.001
CV death, nonfatal MI, or UTVR through 30 days	5.9	7.4	0.784 (0.688,0.894)	<0.001
All cause death, nonfatal MI, or nonfatal stroke through study end	10.2	12.1	0.831 (0.751,0.919)	<0.001
CV death, nonfatal MI, nonfatal stroke or rehospitalisation for cardiac ischemic event through study end	11.7	13.8	0.838 (0.762,0.921)	<0.001
Definite or probable stent thrombosis through study end[a]	0.9	1.9	0.494 (0.361, 0.677)	<0.001

a N=6422 for EFIENT and N=6422 for clopidogrel.

Ancillary analyses

Elderly patients and patients with weight <60 kg

Analyses of the risk for TIMI bleeding by different weight and age cut-offs in study TAAL indicate that the odds ratio for bleeding with 10 mg prasugrel increases around the weight limit of less than 60 kg and age limit of greater than or equal to 75 years. The additional analyses presented suggest an increased bleeding risk associated with weight < 60 kg and age ≥ 75 years and the CHMP raised this as a major objection regarding the proposed reduced 5 mg MD in these two populations.. However, patients over 75 years of age weighing more than 60 kg did not seem to have an increased prasugrel exposure to the same extent as in patients weighing <60kg. This issue was also addressed in the oral explanation held at the CHMP. The results of the analysis conducted *via* a PK/PD model to evaluate the dose 5 mg in patients < 60 kg or ≥ 75 years of age need clinical confirmation and the Company is conducting such studies as part of the follow-up measures..

Comparison of 10-mg Prasugrel Exposure by Weight and Age Categories - Study TAAL

Group	N	Mean Age (years)	Mean Weight (kg)	G-Mean AUC (ng*hr/mL)	Ratio of Geometric Mean (90% CI)[a]
≥60kg and <75years	996	58	85	81.3	
<60kg and <75years	36	60	54	101.9	1.254 (1.105, 1.422)
<60kg and ≥75years	11	80	53	127.5	1.569 (1.252, 1.965)
≥60kg and ≥75years	110	79	78	94.5	1.163 (1.079, 1.253)

Abbreviations: CI = confidence interval; G-Mean = geometric least square mean; N = number of subjects in the specified subgroup.
[a]versus ≥60 kg and <75 years

The question of the proposed reduction in the maintenance dose of prasugrel by one half in elderly patients (> 75 years) to reduce the risk of bleedings while not compromising the efficacy of the drug was discussed by the Scientific Advisory Group requested by the CHMP. This was also addressed at the oral explanation. Based on the increased risk of bleeding in patients ≥ 75 years of age treated with a 10 mg maintenance dose, a very strong wording in the SPC is implemented, stating that use in patients ≥75 years of age is generally not recommended and advising caution for the use of prasugrel in the elderly ≥75 years (i.e. individual benefit/risk evaluation and reduced maintenance dose of 5 mg). Although,the evidence for a 5 mg dose is based only on PK/PD analyses and no clinical data currently exist on the safety of this dose in the very elderly, it is believed that the treatment option in specifically selected and evaluated elderly patients at increased risk for ischemic events should be open after a careful, individual risk benefit evaluation.
In addition, reliable risk minimisation measures must be put in place and safety and efficacy data from clinical trials with this sub-population must be provided to the CHMP, as stated in the list of follow up measures. Adequate educational strategies prepared along with the scientific societies are to be put in place as requested by the CHMP as a condition for the safe and effective use of this medicinal product.

- Clinical efficacy results in special populations

In-stent thrombosis

Applying the ARC definitions (which included angiographic and clinical principles), there was a significant reduction in stent thrombosis in the prasugrel group including both reductions in early (<30 days) and late (≥ 30 days) stent thrombosis that was consistent in the 3 populations (UA/NSTEMI, STEMI, and All ACS). The RRR observed in both UA/NSTEMI and STEMI groups it is stated to be of nearly a 50%. A significant reduction in the rate of the incidence of the primary endpoint was found among patients receiving prasugrel in combination with bare-metal stents (9.37% P vs. 11.59% C) alone and in those receiving prasugrel in combination with at least one drug-eluting stent (8.67% P vs. 10.86% C). A lower incidence in the need of urgent target-vessel revascularization in the prasugrel group was also found.

Previous stroke/TIA

From the multivariate analysis the only risk factors differentially influencing the primary efficacy endpoint for prasugrel compared with clopidogrel were prior TIA or stroke and diabetes (see below). In particular, primary endpoint results in the All ACS population in those that had a prior history of TIA or stroke seem to favour clopidogrel. (prasugrel N 262 n 47 (17.94%) vs clopidogrel N 256 n 35 (13.67%) HR 1.375 CI 95% (0.886, 2.132) p= 0.153). Also, a higher incidence of nonfatal stroke and all stroke either hemorrhagic or non-hemorrhagic, when compared with clopidogrel (nonfatal stroke: 5.73% versus 0.78%, p-value =.002; all stroke: 6.49% versus 1.17%, p-value =.002) was observed. These patients with prior TIA or stroke have now been contraindicated to prasugrel.

Diabetes

For the diabetic population the incidence of the primary efficacy endpoint (All ACS, prasugrel N 1576 n 180 (11.42%) vs clopidogrel N 1570 n 248 (15.80%) HR 0.709 CI 95% (0.582, 0.854) p= 0.001) and each secondary efficacy composite endpoint was lower in subjects randomized to prasugrel compared to subjects randomized to clopidogrel in all 3 populations (UA/NSTEMI, STEMI, and All ACS). Recently published clinical results have suggested that subjects with diabetes may have greater

platelet reactivity and a lower antiplatelet response during clopidogrel treatment. In contrast, the current observations suggest that in subjects with stable CAD (study TABR and TABL), prasugrel treatment provided consistent levels of platelet inhibition in those with and without diabetes. It is proposed that more potent platelet inhibition with prasugrel may result in improved clinical outcomes in ACS subjects with diabetes.

- Discussion on clinical efficacy

Regarding clinical relevance and general interpretation of the results in the context of current evidence, study TAAL could be large enough to address separately the thienopyridine-mediated platelet inhibition in the two major presentation forms of acute coronary syndrome (that is, UA/NSTEMI and STEMI). The median time from onset of qualifying symptoms to randomization in study TAAL in subjects presenting with UA/NSTEMI was 28.9 and 29.0 hours for patients randomized to prasugrel or clopidogrel treatment, respectively. Upper quartiles were 48.6 and 49.0 hours, respectively. In study TAAL, all patients with UA/STEMI were randomized after coronary pathoanatomy was known, i.e. after coronary angiography. The strategy of administering the thienopyridine LD once coronary anatomy is known appears to be preferred because of concerns about surgical bleeding in patients treated with clopidogrel that subsequently undergo CABG surgery. It is acknowledged that the optimum timing of platelet inhibition with a thienopyridine has been debated in recent years. The benefits of the early administration of clopidogrel before PCI does not come from randomised clinical trials primarily aimed to this end, but from post-hoc subgroup analyses and observational studies.

The primary objective of study TAAL was to test the hypothesis that prasugrel co-administered with aspirin was superior to clopidogrel co-administered with aspirin in the treatment of subjects with acute coronary syndromes (ACS) who were to undergo percutaneous coronary intervention (PCI), as measured by a reduction in the composite endpoint of cardiovascular (CV) death, nonfatal myocardial infarction (MI), or nonfatal stroke at a median follow-up of at least 12 months. It was pre-specified that the primary endpoint was analyzed first in subjects with unstable angina and non-ST-segment elevation myocardial infarction. Efficacy superiority of prasugrel has been fully demonstrated for the primary and all secondary endpoints. As mentioned earlier, the timing of prasugrel LD treatment in study TAAL deviated from the present treatment guidelines and this initially raised objection was addressed by presenting several lines of evidence from study TAAL, all of which suggested that the timing of clopidogrel LD did not substantially influence the overall efficacy (or safety) of prasugrel observed in this trial. It was noted that for subjects treated with GPIIb/IIIa inhibitors, there was no evidence that the relative benefit for prasugrel versus clopidogrel was reduced or that there was an excess need for bail out GPIIb/IIIa inhibitor use during PCI in those patients randomised to clopidogrel in the TAAL study. This observation could indirectly indicate that the timing of study drug LD did not substantially influence overall efficacy. Furthermore, when study drug LD is administered before or during PCI, both clopidogrel and prasugrel would be at their near-maximal levels of platelet inhibition achievable by the LD in the early hours following the PCI. This claim is well supported by pharmacokinetic data and *ex vivo* platelet inhibitory activity for prasugrel and clopidogrel, and supports that the timing of study drug LD did not substantially influence the overall efficacy. Stronger evidence for this position originates in the subgroup analysis of patients pre-treated with thienopyridine. In study TAAL, if coronary anatomy was determined or primary PCI for STEMI (≤12 hours) was planned, pre-treatment with study drug was allowed for up to 24 hours before PCI. The percentage of subjects in this pre-treated subgroup reaching the composite endpoint of CV death, nonfatal MI, or nonfatal stroke from randomisation through study end was 9.94 and 11.29, respectively, for subjects pre-treated with prasugrel or clopidogrel. Although not statistically significant for this subgroup, the difference rather strongly favours the notion that that the timing of study drug LD to a large extent did not influence overall efficacy. Additional examination of the subgroup data shows that the predominant benefit of being randomised to prasugrel treatment is not evident in the reduction in peri-procedural MI, a time when prasugrel would presumably have the greatest early advantage, but rather in the reduction in subsequent clinical MI. It is acknowledged that this observation also supports the position that timing of study drug LD is not crucial to the overall efficacy. Finally, the analysis of long-term clinical benefits in Study TAAL confirms the lack of influence of timing of study drug on efficacy. Considering all of these lines of evidence, it is unlikely

that timing of study drug LD had major importance to the overall efficacy demonstrated in Study TAAL.

Regarding the subjects presenting with STEMI, the number and percentage of subjects reaching the primary composite endpoint were 174/1769 (9.84%) and 216/1765 (12.24%) for those randomized to prasugrel or clopidogrel, respectively, (HR=0.793 (95% CI 0.649-0.968)). The treatment benefit was durable; at 3 days, at 30 days, and at study end. Regarding possible effects of prior fibrinolytic treatment, the percentage of subjects reaching the composite endpoint was 6.4% and 8.7% (randomized to prasugrel or clopidogrel, respectively) if fibrinolytic therapy was used before PCI. The corresponding percentages if fibrinolytic therapy was not used were 10.2% and 12.7%. There was no significant statistical interaction between the treatment benefit observed with prasugrel and prior treatment with a fibrinolytic agent in those presenting with STEMI. It is therefore unlikely that the efficacy benefit with prasugrel in subjects presenting with STEMI was influenced by the administration of a fibrinolytic agent prior to PCI. There was a lower incidence of the primary composite endpoint in subjects randomized to prasugrel compared to clopidogrel in the STEMI population undergoing primary (≤12 hours) PCI (10.06 % versus 11.50%; HR=0.872; p=.266). In the STEMI population undergoing delayed (>12 hrs) PCI the corresponding values were 9.40 % versus 13.96%; HR=0.649; p=.015. Initially, these data suggest that patients presenting with STEMI late after symptom onset benefit from prasugrel treatment in particular. However, these patients were in principle handled like patients with UA/NSTEMI, i.e. they were randomised and received study treatment after diagnostic coronary angiography. In addition, the description of the primary endpoint was simplified in the wording of the indication to increase the clarity, but data on each of the individual components of the primary endpoint is retained in the SPC in a relevant section.

For some patients at special risk (very elderly ≥75 years, patients weighing < 60 kg) dose reduction is suggested. After an LD of 60 mg, an MD of 5 mg once daily is recommended, but these patients were not adequately studied with a 5 mg maintenance dose. These considerations formed the basis of a major objection raised by the CHMP. New safety/efficacy analyses based on the active metabolite exposure and PK/PD simulations were presented in the CHMP oral explanation and were conducted in order to support the proposed reduction to a 5 mg prasugrel MD in subjects <60 kg or ≥ 75 years of age. The reduction of the dose is proposed for the following reasons:

- higher exposure to the prasugrel active metabolite in this sub-population associated with an increased risk of bleeding
- bleeding in subjects <60 kg or ≥75 years was predominantly confined to subjects with the highest exposure
- subjects <60 kg or ≥75 years of age with lower exposure to the active metabolite had similar risk of bleeding as subjects ≥60 kg or <75 years of age
- based on the results of a predictive model, reducing the prasugrel MD to 5 mg in subjects <60 kg or ≥75 years of age produces similar exposure as was observed in the lowest quartile exposure in the overall population in study TAAL on the prasugrel 10 mg MD, a quartile of exposure where efficacy was maintained and the risk of bleeding was lowered.

In addition, two dedicated studies aimed to compare the PK, PD, safety, and tolerability of prasugrel in subjects <60 kg or ≥75 years treated with a MD of either prasugrel 5-mg, prasugrel 10-mg, or clopidogrel 75-mg will be conducted as part of the follow up measures. Further a post-authorisation study will be performed to assess the benefit/risk of prasugrel used in real life setting. Results from the study H7T-MC-TABY (TABY) with 10,000 randomised subject assessing the efficacy and safety of prasugrel compared to clopidogrel in medically managed subjects with ACS who have experienced a recent UA/NSTEMI event will be made available to the CHMP and this commitment is part of the follow up measures. The CHMP accepted the proposed dose reduction in patients weighing < 60kg. Although, the dose-reduction in the very elderly ≥75 years and the benefit/risk with the 5 mg MD is not fully clinically supported at present, it is believed that the treatment option in specifically selected and evaluated elderly patients at increased risk for ischemic events should be open after a careful, individual risk benefit evaluation.

Clinical safety

Introduction

The clinical safety evaluation of prasugrel is primarily based on the pre-specified primary database from the pivotal TAAL study. It includes data from 13457 treated subjects with acute coronary syndromes (ACS) undergoing percutaneous coronary intervention (PCI) who were treated with prasugrel (6741 subjects) or clopidogrel (6716 subjects), co-administered with aspirin for up to 15 months. These patients have been exposed to the proposed prasugrel dosing regimen (60-mg loading dose [LD]/10-mg maintenance dose [MD]). The secondary safety database includes data from the 4 smaller studies; TAAD, TAAH, TABL, TABR grouped into "All but TAAL (ABT)", and Study TAAL limited to the first 30 days after first dose of study drug, "TAAL-30". The tertiary safety database comprises the safety data from clinical pharmacology studies. Two major populations contributed to the All ACS population:

- UA/NSTEMI (clinical presentations being UA or NSTEMI within 72 hours)
- STEMI (clinical presentations being STEMI ≤12 hours since symptoms onset or STEMI >12 hours since symptoms onset)

The number of subjects with STEMI was capped at 3534 subjects. The majority of subjects in the All ACS population were male (74%) and Caucasian (92%). The mean age was 61 years and the mean weight was 83 kg. The geographic region of origin was Europe in approximately 50% and North America in 32%.

- Patient exposure

A total of 8656 subjects (6741 from the primary safety database, 940 from the secondary safety database and 975 from the tertiary safety database) have been exposed to at least 1 dose of prasugrel across all completed clinical and clinical pharmacology studies. The overall exposure to prasugrel in the primary safety database was 6483 subject-years. More than half of the subjects treated with prasugrel were exposed for more than 1 year. For the primary safety database "while at risk" was defined as the period from first study drug administration through 7 days after permanent study drug discontinuation (the termination visit) or through 464 days after randomisation, whichever came first.

- Adverse events

In the primary safety database, treatment-emergent adverse events (TEAEs) were reported in 80.34% of prasugrel treated patients and 80.02% of clopidogrel treated patients. Clinically significant AEs were also pre-specified apart from CEC adjudicated bleeding endpoints, and included AEs of particular interest (thrombocytopenia, thrombotic thrombocytopenic purpura [TTP], neutropenia, leucopenia, pancytopenia, torsades de pointes/QT prolongation, allergic reactions, and abnormal hepatic function). No statistically significant difference between treatments was observed for these clinically significant TEAEs (not either for TEAEs and SAEs) when the analysis was limited to the first 3 days (prasugrel 3.16%, clopidogrel 2.75%) or the first 30 days (prasugrel 5.34%, clopidogrel 5.0%). The majority of drug related adverse events were related to bleeding and the risk was higher with prasugrel than with clopidogrel.

Hemorrhagic AEs occurred with a statistically significant higher incidence in the prasugrel treated patients compared to clopidogrel, 29.70% *vs* 22.04% ($p<0.001$). Both CEC- adjudicated non-CABG-related TIMI Major Bleeding, TIMI Major or Minor Bleeding and TIMI Major, Minor, or Minimal Bleeding were statistically significantly increased in prasugrel vs. clopidogrel patients (2.17% vs. 1.65%, 4.49% vs. 3.44%, and 10.86% vs. 7.86%, respectively). Though numbers of patients undergoing CABG were small (213 in the prasugrel group, 224 in the clopidogrel group), it appeared that the risk of CABG related TIMI Major or Minor Bleeding was approximately tripled in the prasugrel arm (30 patients [14.08%] vs. 10 patients [4.46%], $p<0.001$). The overall distribution of hemorrhagic TEAEs and the higher incidence of hemorrhagic TEAEs in prasugrel and clopidogrel treated patients, respectively, was comparable between the UA/NSTEMI subgroup (prasugrel 29.95%, clopidogrel 22.21%), and the STEMI subgroup (prasugrel 28.97%, clopidogrel 21.54%). Nevertheless, although the number of STEMI patients receiving fibrinolytic treatment was small, the bleeding events were comparable between STEMI patients who received fibrinolytic treatment and the patients who did not receive fibrinolytic treatment. In addition, the efficacy benefit seen with prasugrel treatment in

the STEMI population, was not outweighed by bleeding complications in STEMI sub-population of patients managed with delayed PCI. In order of descending frequency, contusion, haematoma, epistaxis, ecchymosis, vessel puncture site haematoma, puncture site haemorrhage, haematuria, and GI haemorrhage were the common (≥1%) hemorrhagic ADRs associated with prasugrel therapy. In the secondary safety database, the overall incidence of hemorrhagic events was higher in ABT versus TAAL-30 for both treatment groups (ABT: prasugrel 35.74%, clopidogrel 20.66%; TAAL-30: prasugrel 18.29%, clopidogrel 14.34%). This was primarily due to the incidence of catheter site haematoma, catheter site haemorrhage, contusion, and epistaxis.

Non-haemorrhagic AEs occurred with a similar frequency in the two treatment groups (77.33% with prasugrel and 77.86% with clopidogrel); statistically significant differences were seen for coronary revascularisation, fatigue, MI, constipation, musculoskeletal pain, cardiac failure (more frequent with clopidogrel) and for pyrexia and tendency to bruise (more frequent with prasugrel). The incidence of infections was similar between the two treatment groups. It is believed that the observed differences in the incidence of pyrexia are in part due to the higher incidence of bleeding in subjects treated with prasugrel, who more frequently received transfusions. Additionally, the causality relationship between rash and prasugrel could not be excluded. The issues have been adequately addressed in the RMP as a potential risk and pharmacovigilance measures will be implemented.

Colon cancer was an uncommon TEAE (0.17% with prasugrel, 0.03% with clopidogrel) that occurred with a statistically significant higher incidence (p=.013) in subjects treated with prasugrel. Of the 19 reports from the prasugrel group, 10 were diagnosed as a result of a diagnostic procedure following a gastrointestinal bleeding. On the basis of these findings, it was concluded that colon cancer was diagnosed more often in subjects treated with prasugrel due to a higher rate of bleeding associated with this therapy.

- Serious adverse event/deaths/other significant events

Deaths

The overall incidence of all-cause deaths was similar between the treatment groups in the primary database (clopidogrel 2.90%, prasugrel 2.76%). The majority of deaths were cardiovascular deaths (prasugrel 1.95% *vs* clopidogrel 2.21%). In the UA/NSTEMI subpopulation, a numerically higher overall mortality was observed in the prasugrel treatment group compared to clopidogrel. However, the explanation is acceptable that the observed numerical increase in overall mortality in prasugrel-treated patients with UA/NSTEMI (9 more deaths) cannot be disentangled from the recognised increased risk of bleeding associated with prasugrel. Elderly patients constitute an especially sensitive population regarding bleeding risk, and explain most, if not all, of the numerically differences in mortality observed in the UA/NSTEMI population.

There was a higher incidence of deaths due to haemorrhage in prasugrel treated patients (both ICH (prasugrel 9 (0.13%) *vs* clopidogrel 5 (0.07%) and non-ICH (prasugrel 9 (0.13%) *vs* clopidogrel 1 (0.01%)) in the All ACS population (see below). The SPC was revised to state the increased risk of major, life-threatening and fatal bleeding associated with the use of prasugrel as compared to clopidogrel in the UA/NSTEMI and All ACS populations.

Summary of Deaths in Study TAAL; All Randomized All ACS Subjects

Deaths All ACS Population	Prasugrel N=6813 n (%)[a]	Clopidogrel N=6795 n (%)[a]	Total N=13608 n (%)[a]
Deaths during study period[b]	188	197	385
Deaths in treated subjects	181	186	367
Deaths in subjects not treated with study drug	7	11	18
Deaths outside of the study period[b]	3	0	3
All Total Deaths[c]	191	197	388
Clinical Endpoints Committee Adjudicated Deaths			
All Cause Death[c]	188 (2.76)	197 (2.90)	385 (2.83)
Cardiovascular Death	133 (1.95)	150 (2.21)	283 (2.08)
Atherosclerotic Vascular Disease[d]	0	3 (0.04)	3 (.0002)
CHF/Cardiogenic Shock	31 (0.46)	30 (0.44)	61 (0.45)
Directly related to revascularization[e]	15 (0.22)	16 (0.24)	31 (0.23)
Dysrhythmia	4 (0.06)	7 (0.10)	11 (0.08)
Pulmonary Embolism	3 (0.04)	0	3 (0.02)
Myocardial Infarction	24 (0.35)	36 (0.53)	60 (0.44)
Sudden or Unwitnessed	36 (0.53)	42 (0.62)	78 (0.57)
Intracranial Hemorrhage	9 (0.13)	5 (0.07)	14 (0.10)
Non-Hemorrhagic Stroke	5 (0.07)	6 (0.09)	11 (0.08)
Other Cardiovascular	6 (0.09)	5 (0.07)	11 (0.08)
Non-Cardiovascular Death	55 (0.81)	47 (0.69)	102 (0.75)
Accidental/Trauma	4 (0.06)	4 (0.06)	8 (0.06)
Nonintracranial Hemorrhage	9 (0.13)	1 (0.01)	10 (0.07)
Infection	11 (0.16)	10 (0.15)	21 (0.16)
Malignancy	21 (0.31)	17 (0.25)	38 (0.28)
Suicide	3 (0.04)	2 (0.03)	5 (0.04)
Other Non-Cardiovascular	7 (0.10)	13 (0.19)	20 (0.15)

Abbreviations: ACS = acute coronary syndromes; CHF = coronary heart failure; PCI = percutaneous coronary intervention; UA = unstable angina.

[a] % is percentage of randomized subjects.

[b] Study period = from randomization through a subject's study termination or 464 days from randomization, whichever was earlier.

[c] There are a total of 388 deaths during the study, with 3 deaths occurring outside the study period, which were listed in the row of "Deaths outside of study period." Therefore, "All total deaths" and "All Cause deaths" differ by 3 subjects.

[d] Atherosclerotic vascular disease excludes deaths from coronary vascular disease.

[e] Death is directly related to hemorrhagic or non-hemorrhagic complications of revascularization (CABG or PCI).

Fatal haemorrhages

Overall, in the All ACS population fatal hemorrhagic events (including CABG-related and Non-CABG-related bleeding events) occurred in 24 subjects (0.36%) in the prasugrel treatment group and 6 subjects (0.09%) in the clopidogrel treatment group. The majority (21/24 deaths in prasugrel patients, 5/6 deaths in clopidogrel patients) were non CABG related TIMI major bleedings during the at risk period. Non CABG-related spontaneous intracranial and gastrointestinal (GI) bleedings were predominant (prasugrel: spontaneous fatal bleedings in 16 patients, hereof 8 intracranial and 6 GI bleedings; clopidogrel: spontaneous fatal bleedings in 4 patients, 4 intracranial and 1 GI bleeding). Non-CABG related instrumented fatal bleedings were only seen in the prasugrel group (4 patients). The incidence of fatal bleedings was also statistically significant in the UA/NSTEMI prasugrel group. The same pattern was observed in the STEMI population but due to insufficient data the statistics could not be evaluated (prasugrel: 7 patients, 0.40%, clopidogrel 2 patients 0.12%). There were no fatal hemorrhagic events in the secondary and tertiary databases.

SAEs

Serious adverse events occurred in 24.70% of prasugrel and 24.26% of clopidogrel treated patients in the primary database. The incidence of non-hemorrhagic SAEs was similar in the prasugrel (22.48%) and the clopidogrel (22.80%) group. Most frequent were non-cardiac chest pain, coronary artery re-

stenosis, chest pain and angina pectoris. Three non-haemorrhagic SAEs that occurred differentially among treatment groups were the higher incidence in the prasugrel group of colon cancer, hypotension, and respiratory failure (which was finally related to the simultaneous occurrence of blood loss). The incidence of hemorrhagic SAEs in the All ACS population while at risk was statistically significantly higher in subjects treated with prasugrel when compared to subjects treated with clopidogrel. Likewise, in the UA/STEMI group the incidence of hemorrhagic SAEs with prasugrel was statistically significantly higher (prasugrel 6.08% *vs* clopidogrel 4.06%). In the STEMI population the incidence was numerically higher though, not statistically different (5.34% vs. 4.26%). Non-CABG related TIMI Major Bleedings (including life threatening and fatal bleedings), TIMI Major or Minor, and TIMI Major, Minor, or Minimal Bleedings were all significantly higher in the prasugrel treated subjects compared to the clopidogrel treated subjects in the All ACS population.

Incidence of Bleeding Events—Clinical Events Committee Adjudicated – Primary Safety Database (Study TAAL) All ACS

Bleeding Events[a]	Prasugrel (N=6813) n (%)	Clopidogrel (N=6795) n (%)	Total (N=13608) n (%)	Hazard Ratio (95% CI)[b]	p-Value[c]
All ACS	6741	6716	13457	NE	NE
Non-CABG-related					
TIMI Major	146 (2.17)	111 (1.65)	257 (1.91)	1.315 (1.028, 1.683)	.029
Life-Threatening	85 (1.26)	56 (0.83)	141 (1.05)	1.517 (1.083, 2.126)	.015
Fatal	21 (0.31)	5 (0.07)	26 (0.19)	4.191 (1.580, 11.113)	.002
Symptomatic ICH	19 (0.28)	17 (0.25)	36 (0.27)	1.119 (0.582, 2.151)	.736
IV Inotrope Required	21 (0.31)	8 (0.12)	29 (0.22)	2.617 (1.159, 5.908)	.016
Surgery Required	19 (0.28)	19 (0.28)	38 (0.28)	0.998 (0.528, 1.885)	.995
Transfusion of≥4 Units	45 (0.67)	30 (0.45)	75 (0.56)	1.499 (0.945, 2.379)	.084
Instrumented	45 (0.67)	38 (0.57)	83 (0.62)	1.182 (0.767, 1.820)	.447
Spontaneous	92 (1.36)	61 (0.91)	153 (1.14)	1.508 (1.091, 2.085)	.012
TIMI Minor	164 (2.43)	125 (1.86)	289 (2.15)	NE	NE
TIMI Major or TIMI Minor	303 (4.49)	231 (3.44)	534 (3.97)	1.314 (1.107, 1.559)	.002
TIMI Minimal	460 (6.82)	314 (4.68)	774 (5.75)	NE	NE
TIMI Major, Minor, or Min	732 (10.86)	528 (7.86)	1260 (9.36)	1.400 (1.252, 1.566)	<.001
Any Transfusion Required[d]	244 (3.62)	182 (2.71)	426 (3.17)	1.34 (1.11, 1.63)	.003
CABG-related	(N=213)	(N=224)	(N=437)		
TIMI Major or Minor	30 (14.08)	10 (4.46)	40 (9.15)	3.587 (1.702,7.557)[e]	<0.001
Fatal	2 (0.94)	0	2 (0.46)	NE	NE

Abbreviations: ACS = acute coronary syndromes; CABG = coronary artery bypass graft surgery; CI = confidence interval; HR = hazard ratio; ICH = intracranial hemorrhage; IV = intravenous; Min = minimal; N = number of subjects in the specified subgroup; n = number of subjects within the specified subgroup reaching the endpoint; NE = not evaluated due to insufficient sample size; NSTEMI = non-ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction; TIMI = Thrombolysis in Myocardial Infarction; UA = unstable angina.

a Subjects experiencing multiple bleeding events may be included in more than one category.

b HR and two-sided 95% CI derived using Cox proportional hazards model.

c Two-sided log-rank p-value based on time to first event analysis compares the event free survival distributions for prasugrel and clopidogrel. Clinical presentation, UA/NSTEMI versus STEMI, was used as a stratification factor in analyses of All ACS subjects.

d Bleeding requiring any transfusion (whole- or packed-blood).

e Odds ratio is based on the frequency procedure. Two-sided p-values are based on Cochran-Mantel-Haenszel general association test with clinical presentation as a blocking factor in All ACS.

In the All ACS population, the significantly higher incidence of TIMI Major bleedings in subjects treated with prasugrel was related to higher rates of GI bleeding (prasugrel 0.93% vs. clopidogrel 0.64%), surgical site bleeding (0.15% vs. 0.01%), and bleeding at other sites not pre-specified or unknown (0.13% vs. 0.01%). A higher incidence of retroperitoneal bleeding was also observed in subjects treated with prasugrel (0.21% vs. 0.12%). Intracranial hemorrhages and puncture site bleeding events were similar between treatment groups (0.28% vs. 0.25% and 0.42% vs. 0.45%, respectively).

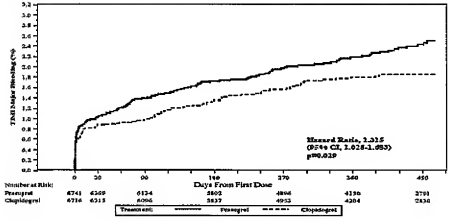
Non-CABG-related TIMI Major and TIMI Life-Threatening bleeding events through 3 days after the first dose of study: The incidence in the All ACS population was numerically higher in subjects treated with prasugrel compared to clopidogrel (prasugrel 0.74% vs. clopidogrel 0.61% for TIMI major and 0.43% vs. 0.31% for Life threatening bleeding), primarily related to a numerically higher incidence of GI bleeding which was reflected by a higher incidence of spontaneous bleeding events. Likewise, in the UA/NSTEMI population, there were numerically more spontaneous and instrumented, bleeding

events in subjects treated with prasugrel compared to clopidogrel through 3 days. In the STEMI population there were no relevant differences between the 2 treatment groups.

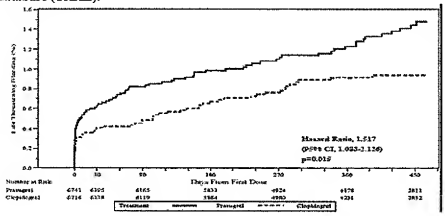
Non-CABG-related TIMI Major and TIMI Life-Threatening bleeding events beyond 3 days after the first dose of study: The incidence was statistically significantly higher in subjects in the All ACS population treated with prasugrel compared to clopidogrel (1.45% vs. 1.05% for TIMI major, 0.84% vs. 053% for TIMI life threatening bleeding), primarily driven by a numerically higher incidence of GI bleeding (0.78% *vs* 0.59%), surgical site bleeding (0.10% *vs* 0.02%), and bleeding at sites not pre-specified or unknown (0.10% *vs* 0%). A higher numerical incidence of retroperitoneal bleeding (0.09% *vs* 0.03%) and surgical site bleeding events (0.10% vs. 0.02%) was observed with prasugrel compared to clopidogrel, which resulted in a statistically significant higher incidence of spontaneous bleeding events (1.14% *vs* 0.78%) and a numerically higher incidence of instrumented bleeding events (0.19% *vs* 0.12%) with prasugrel compared to clopidogrel. For the subcategory of TIMI Life-Threatening bleeding, subjects treated with prasugrel had a statistically significant higher incidence of fatal bleeding (0.24% *vs* 0.06%) and a numerically significant higher incidence of bleeding requiring intravenous inotropic medication in addition to multiple transfusion units. A higher incidence in Non-CABG-related TIMI Life-Threatening bleeding events was observed in the UA/NSTEMI population, but not in the STEMI population.

The time course of TIMI major bleeding events and its subgroup Life threatening bleedings is shown in the following figures.

Kaplan-Meier estimates of the incidence of Non-CABG related TIMI major bleeding events while at risk—CEC adjudicated for all treated All ACS subjects in primary safety database (TAAL).



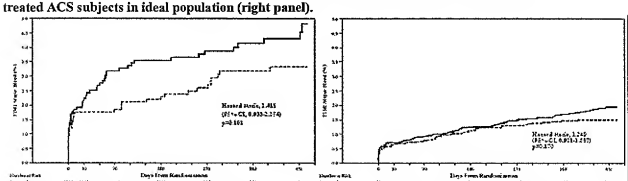
Kaplan-Meier estimates of the incidence of Non-CABGrelated TIMI life-threatening bleeding events while at risk— CEC adjudicated for all treated All ACS subjects in primary safety database (TAAL).



The Kaplan Meier curves separated early for Non-CABG-related TIMI Major and TIMI Life-Threatening bleeding events while at risk, favouring clopidogrel. Another separation is seen beyond 1 year and potentially also at around 30 days. Multivariate analyses identified independent risk factors for increased occurrence of Non-CABG-related TIMI Major bleeding. These were prasugrel treatment, weight < 60 kg, age ≥ 75 years, history of hypertension, history of prior TIA or stroke, and use of GPIIb/IIIa inhibitor (from symptom onset through 3 days after randomization).

Further analyses which demonstrated that mainly patients at risk (patients with a prior history of TIA/stroke, patients with body weight < 60 kg and patients ≥ 75 years of age) are responsible for the unfavourable safety profile of prasugrel, were provided during the evaluation. The figures below display the Kaplan Meier curves for Non-CABG related TIMI Major bleeding events in the "ideal population" (excluding subjects with a history of TIA or stroke, subjects ≥75 years of age taking a MD of 10 mg/day, or subjects <60 kg taking a MD of 10 mg/day) and in the non-ideal population (subjects with history of TIA or stroke, ≥75 years of age or weighing <60 kg). The difference between prasugrel and clopidogrel between 30 and 90 days, is explained by the higher bleeding risk in subjects in the non-ideal population. However, major bleedings also appear to accrue beyond 1 year. The CHMP therefore requested to limit the treatment duration with prasugrel to 12 months, consistent with the existing treatment duration recommendation for clopidogrel in this clinical setting.

Kaplan-Meier estimates of the incidence of non-CABG-related TIMI Major bleeding events while at risk – CEC adjudicated treated ACS subjects in non-ideal population (left panel) and treated ACS subjects in ideal population (right panel).



Considering the ideal population only, the rate of Non-CABG-related TIMI Major and Life-Threatening bleeding in prasugrel treated subjects was numerically, but not statistically higher compared to clopidogrel-treated subjects. The number of fatal bleeding events beyond 3 days of treatment was 7 in prasugrel-treated subjects versus 2 in clopidogrel-treated subjects, as compared to 21 versus 5 in the All ACS population for the entire treatment duration ("at risk period"). Three of the 7 fatal bleeding events in prasugrel-treated subjects resulted from procedural complications, while 4 were fatal ICH (1 traumatic). Both clopidogrel fatal events were spontaneous ICH. Consequently, the number of spontaneous fatal bleeding events beyond 3 days during MD was similar between treatment groups. During the first 3 days, CABG and non-CABG related fatal bleedings were as follows: one fatal bleeding occurred in the clopidogrel group and 6 fatal bleedings (3 each from UA/NSTEMI and STEMI) occurred in the prasugrel group. Four of these patients belonged to the ideal population, and 3 of these had an instrumented or traumatic bleeding. Major bleeding may increase the short-term risk of ischemic events and the risk of death. This was evident in study TAAL. In addition, it was shown that for the All ACS population and both treatment groups together, there was no statistically significant difference between treatment groups for the risk of ischemic events (CV death, CV death/MI, CV death/MI/stroke) beyond and within 30 days in patients having experienced no major bleed versus patients having experienced a major bleed, although the risk was numerically higher for those with major bleed. A comparison of all-cause deaths within and beyond 30 days by treatment group and clinical presentation (UA/NSTEMI, STEMI) shows that mortality within 30 days is roughly comparable for prasugrel and clopidogrel (for the STEMI population even numerically slightly more favourable for prasugrel) if patients did not have a TIMI major bleeding. However, all cause death in patients with a TIMI major bleeding and treated with prasugrel was higher when compared to patients treated with clopidogrel (all ACS population 15.6% vs. 10.9% (p=0.06), similarly in the UA/NSTEMI and STEMI subgroups). Analysis of the "ideal population" shows that all cause death is still numerically higher for prasugrel compared to clopidogrel but the difference is not statistically significant (all ACS population 9.4% vs 7.3%, p=0.52). These deaths are predominantly attributable to fatal bleedings and the increased bleeding risk with prasugrel is clearly stated in the SPC. Beyond 30 days, mortality is comparable for prasugrel and for clopidogrel treated patients not having had a major bleed. For patients with major bleed, events are too few to draw firm conclusions. .

- Laboratory findings

The safety data suggests that prasugrel therapy is not associated with clinically significant thrombocytopenia, neutropenia, or leucopenia. The number of subjects with normal Hct, Hgb, or RBC at baseline, and abnormally low values at any time post-baseline, was statistically significantly higher in subjects treated with prasugrel compared to clopidogrel and probably reflects the higher incidence of bleeding events. Hepatotoxicity of prasugrel is not suggested by the laboratory data.

- Safety in special populations

Renal impairment: In total, 707 patients in the prasugrel group and 769 in the clopidogrel group had a creatinine clearance ≤ 60 ml/min (measured by Cockroft-Gault formula). For both treatments (prasugrel *vs* clopidogrel) a higher incidence of TIMI major or minor bleeding events was observed in patients with creatinine clearance ≤ 60 ml/min compared to patients with normal renal function (9.48% and 6.76%, p=0.052 *vs* 3.89% and 2.98%). The same tendency was observed for life threatening bleeding events (2.26% and 1.04%, p= 0.059 vs. 1.09% and 0.78%). Multivariate analysis did not identify renal impairment as expressed by creatinine clearance as a predictor of higher bleeding risk. More than half of patients with creatinine clearance <30ml/min were also very elderly patients. When analysing bleedings excluding these patients >75 years, the bleeding risk was still elevated but not significantly different between prasugrel and clopidogrel.

Hepatic impairment: Active metabolite exposures in subjects with moderate hepatic impairment and in healthy subjects are comparable. Patients with severe hepatic impairment were excluded from TAAL and a contraindication for these patients is included in the SPC. There were only a limited number of patients with less severe forms of hepatic impairment included in TAAL. Although safety did not seem to be compromised in these patients, safety conclusions must be drawn cautiously.

Age: In total, 901subjects in the prasugrel group and 908 subjects in the clopidogrel group were ≥ 75 years. In both treatment groups, twice as many subjects ≥75 years experienced Non-CABG-related

TIMI major or minor- (prasugrel 8.98%, clopidogrel 6.94%) as well as life-threatening bleeding events (prasugrel 2.58%, clopidogrel 1.57%) compared to patients below the age of 75 years. This was observed for both treatment groups. Furthermore, for prasugrel-treated patients ≥75 years (UA/NSTEMI as well as All ACS), twice as many experienced any stroke compared to clopidogrel treatment (2.89% *vs* 1.43%). A statistically significant difference was seen on the incidence of fatal bleeding in favour of clopidogrel. Use of prasugrel in this patient population is generally not recommended. If prescribed after a careful individual risk/benefit evaluation, a lower MD of prasugrel 5 mg should be used (please refer to section on Clinical efficacy). Further information on the 5-mg dose in this population will be obtained from future or ongoing clinical studies.

Prior Transient Ischemic Attack or Stroke: In total, 262 subjects of 6484 in the prasugrel group and 256 subjects of 6464 in the clopidogrel group had a prior history of TIA or stroke. Subjects with a history of prior TIA or stroke and treated with prasugrel had a statistically significant higher incidence of nonfatal stroke (15/262 (5.73%) vs. 2/256 (0.78%), p<0.001) and all stroke (both, fatal and nonfatal, hemorrhagic or non-hemorrhagic) (17/262 (6.49%) vs. 3/256 (1.17%), p<0.001), when compared to clopidogrel. A similar, statistically significant pattern was observed in the UA/NSTEMI population, whereas the number of stroke events in the STEMI population with prior history of TIA/stroke was too low to allow any reliable conclusions. In addition, a history of prior TIA/stroke in the all ACS population was associated with a higher risk of Non-CABG-related TIMI Major or Minor bleeding events (Prasugrel: 20/257 (7.78%) vs. clopidogrel: 10/252 (3.97%), p=0.054) and of Non-CABG-related TIMI Major Life-Threatening bleeding events (11/257 (4.28%) vs. 3/252 (1.19%), p=0.026, including fatal bleeding and symptomatic ICH) with prasugrel therapy. Regarding the fatal ICH, 2 out of 9 patients in the prasugrel group had a prior history of TIA/stroke *vs* 0 out of 5 patients in the clopidogrel group. The higher bleeding risk in patients with prior TIA or stroke was not associated with higher exposure during MD. Thus, patients with a prior history of TIA or stroke are contraindicated for prasugrel.

Low body weight: The risk of Non-CABG-related TIMI Major or Minor bleeding events for patients weighing below 60 kg was greater for prasugrel treated patients compared to clopidogrel treated, though not significantly different. However, the number of patients weighing less than 60 kg was very low (664 subjects in total in both treatment groups). PK data has shown that the active metabolite exposure increases as body weight decreases (see section on clinical pharmacology). A lower prasugrel 5 mg MD for this subgroup could be used. A PK/PD study to investigate the 5 mg dose in this patient population will be conducted as part of the FUMs.

Ethnicity: Prasugrel active metabolite exposure in Asian subjects was 43% higher after a 60-mg prasugrel LD and 40% higher during 10-mg prasugrel MD compared to Caucasian subjects. Based on point estimates for comparisons between Asians and Caucasians, body weight accounted for about one-third of the exposure difference between the two ethnic groups. There were so few Non-CABG-related Major or Minor bleeding events in the non-Caucasian populations that a meaningful comparison could not be done. A new PK/PG study in approximately 715 Asian ACS subjects in order to clarify which could be the optimal dose for this population will be conducted. Until this data become available a cautious approach is appropriate by including a warning in the SPC.

- Safety related to drug-drug interactions and other interactions

Specific *in vivo* drug-interaction studies were conducted with prasugrel and aspirin, ketoconazole (a potent CYP3A inhibitor), rifampicin (a potent inducer of CYP3A and CYP2B6 and an inducer of CYPs 2C9, 2C19, and 2C8), atorvastatin (a statin metabolized by CYP3A), warfarin (an anti-coagulant metabolized by CYPs 2C9 and 2C19), heparin, bupropion (a CYP2B6 substrate), and digoxin (a P-glycoprotein [P-gp] substrate). The effect of smoking and alcohol consumption were also evaluated across clinical pharmacology studies. Overall, these analyses detected no clinically relevant drug interactions.

Proton pump inhibitors may slow the rate, but not the extent, of appearance of prasugrel's active metabolite in plasma. Prasugrel can be co-administered with a proton pump inhibitor (PPI) or a H2-receptor antagonist. However, the SPC was revised to state that administration of the loading dose without co-administration with PPI may provide most rapid onset of action.

- Discontinuation due to adverse events

In the All ACS populations, overall incidence of study drug discontinuation due to treatment-emergent adverse events (TEAEs) was higher in subjects treated with prasugrel (462/6741 (7.15%)) compared to clopidogrel (390/6716 (6.02%)). The rate of discontinuation of study drug due to an AE was similar between treatment groups through 90 days, at which time the Kaplan-Meier curves diverge in favour of clopidogrel. The higher incidence of study drug discontinuation with prasugrel due to AE was primarily due to the higher incidence of hemorrhagic events (with GI hemorrhage (33/3741 (0.49%) vs. 21/6716 (0.32%)) and epistaxis (0.31% vs. 0.12%) being the most common). Atrial fibrillation (20/6741 (0.31%) vs. 33/6716 (0.51)) and rash (0.28% vs. 0.42%) were the non-hemorrhagic events leading to the highest incidence of permanent study drug discontinuation, but the rate between the 2 treatment groups was similar. In the secondary database there were no observed treatment differences between prasugrel and clopidogrel in the incidence of SAEs and non-serious TEAEs leading to premature discontinuation of study drug.

- Post marketing experience

There is currently no post-marketing experience with the use of this product.

- Discussion on clinical safety

The key safety findings associated with prasugrel treatment were a statistically higher incidence of hemorrhagic AEs. Adjudicated non-CABG-related TIMI Major Bleeding, TIMI Major or Minor Bleeding and TIMI Major, Minor, or Minimal Bleeding were statistically significantly increased in the prasugrel group compared to the clopidogrel group as well as fatal hemorrhagic AEs which were also higher in the prasugrel group. The Kaplan-Meier curves separated early for Non-CABG-related TIMI Major and TIMI Life-Threatening bleeding events while at risk, favouring clopidogrel. It seems that the curves remained parallel between 90 and 360 days. However, between 30 and 90 days as well as beyond 360 days, events continued to increase in subjects treated with prasugrel while a diminished accrual rate was seen in subjects treated with clopidogrel. Patients for whom a 10 mg maintenance dose is not recommended (those with a history of TIA or stroke – contraindication- , subjects ≥75 years of age taking a MD of 10 mg/day, or subjects <60 kg taking a MD of 10 mg/day) are responsible for the accrual between 30 and 90 days. The apparent accrual beyond 350 days cannot be explained. At the same time, the clinical benefit of prasugrel beyond 12 months seems not sufficiently supported by clinical data, this is why the CHMP has recommended that dual antiplatelet therapy with prasugrel should be restricted to 12 months treatment duration, in line with current clinical recommendations for dual antiplatelet therapy. This was also accepted by the Scientific Advisory Group of the CHMP.

The observation that fatal bleedings were higher in the prasugrel group compared to the clopidogrel group was of concern. However, the number of the spontaneous fatal bleeding events was similar between treatment groups, especially considering the ideal population for whom the prasugrel 10-mg MD would be recommended (subjects without a history of TIA or stroke, subjects ≥75 years of age, or subjects <60 kg). The rate of Non-CABG-related TIMI Major or Minor bleeding events in the ideal population was not statistically different between treatment groups but numerically higher for prasugrel. Though numbers of patients undergoing CABG were small, the risk of CABG related TIMI Major or Minor Bleeding was approximately tripled in the prasugrel arm, in particular in patients undergoing CABG within 7 days of the last dose of study drug. Bleeding events were also the primary reason for treatment discontinuations.

Subgroups associated with a statistically significant increase in Non-CABG-related TIMI Major bleeding were: ≥75 years old, body weight <60 kg, and history of prior TIA or stroke. The lastly mentioned patients have been contraindicated. For patients < 60 kg the events were associated with higher exposure of the active metabolite, supporting the proposed prasugrel 5-mg MD in this subgroup. For patients ≥ 75 years the events were partly associated with increased exposure to the active metabolite along with a greater susceptibility to bleeding. A dose adjustment strategy was justified by further analyses and planned future clinical studies for both the low weight patients and the very elderly. The use of prasugrel in patients ≥ 75 years of age is generally not recommended. If, after a careful individual benefit/risk evaluation by the prescribing physician, treatment is deemed necessary in the ≥ 75 years age subgroup then following a 60 mg loading dose, a reduced prasugrel 5 mg MD should be prescribed.

The evidence for the 5 mg MD is based only on PK/PD analyses and no clinical data currently exist on the safety of this dose in the ≥ 75 years age group.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan, which included a risk minimisation plan.

Table Summary of the risk management plan

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Identified Risks		
1. Haemorahage: (Intracranial haemorrhage, Gastrointestinal haemorrhage, Intraocular haemorrhage Percutaneous Coronary Intervention-Related Haemorrhage, Coronary Artery Bypass Graft-Related Haemorrhage Other Procedure-Related Haemorrhage, Epistaxis)	• Routine pharmacovigilance: monitor AEs and SAEs through routine clinical trial and spontaneous post-marketing surveillance. • Targeted surveillance for specific AEs preidentified for targeted follow-up. • In-hospital registry to monitor prasugrel use and bleeding risk during the index hospitalisation compared to clopidogrel in a real life EU clinical setting.	• Contraindication for patients with history of stroke or transient ischaemic attack (TIA) and for patients with active pathological bleeding in Section 4.3 of SPC. • Section 4.2: dose adjustment for patients with risk factors for increased risk of bleeding: patients ≥ 75 years of age and patients <60 kg. • Wording in sections 4.2 and 4.4 of the SPC regarding the restricted use of EFIENT in patients ≥ 75 years of age, and maintenance dose reduction in these patients. • Caution for patients with a propensity to bleed, and with risk factors for an increased risk of bleeding (section 4.4) • Caution with concomitant administration of medicinal products that may increase the risk of bleeding (section 4.4) • Further recommendations to minimise the risk of haemorrhage, including CABG-related haemorrhage, are given in Section 4.4 (Surgery) and Section 4.8 of the SPC. Section 4.4 of the SPC recommends discontinuation of EFIENT at least 7 days prior to surgery. • Epistaxis is listed as ADR in Table 2, Section 4.8 of SPC. • Additional risk minimisation for patients ≥ 75 years of age will be provided by health care professional education to ensure that the information and recommendations in the SPC are adequately communicated. The MAH commits to work with scientific societies to develop educational vehicles for this purpose.
2. Anaemia	• Routine pharmacovigilance: monitor AEs and SAEs through routine clinical trial and spontaneous post-marketing surveillance. • Targeted surveillance for specific AEs preidentified for targeted follow-up.	• Listed as ADR in Table 2, Section 4.8 of SPC.

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Potential Risks		
Risks associated with off-label use	• Routine pharmacovigilance: monitor AEs and SAEs through routine clinical trial and spontaneous post-marketing surveillance. • Targeted surveillance for specific AEs preidentified for targeted follow-up. • In –hospital registry to monitor prasugrel use and bleeding risk during the index hospitalisation compared to clopidogrel in a real life EU clinical setting. • Off-Label Use in Patients Post-Discharge: To monitor the off-label use post-discharge in patients treated with prasugrel. The databases will capture data pertaining to drug utilisation to monitor in what patients prasugrel is used, and at what doses.	• Not in proposed SPC as not confirmed signal.
Phototoxicity (Skin or Ocular)	As bullet 1 above.	• Not in proposed SPC as not confirmed signal.
Drug-Induced Hepatic Injury	As bullets 1 and 2 above.	• Not in proposed SPC as not confirmed signal.
Allergic Reactions	As bullets 1 and 2 above.	• Not in proposed SPC as not confirmed signal.
Thrombocytopenia	As bullets 1 and 2 above.	• Not in proposed SPC as not confirmed signal.
Neutropenia	As bullets 1 and 2 above.	• Not in proposed SPC as not confirmed signal.
Thrombotic Thrombocytopenic Purpura	As bullets 1 and 2 above.	• Warning in Section 4.4 of the SPC includes a description of this serious event.

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Missing Information		
Concomitant use with fibrinolytics, clopidogrel, and chronic use of NSAIDs (non ASA).	• Continue to analyse AE reports in clinical trials • Periodically review and analyse safety database • Any spontaneously reported case associated with an exposure condition (EC) is managed according to internal procedures for clarification. • Safety surveillance intends to identify signals associated with the ACS subpopulations and use of drugs associated with an increased risk of bleeding. Concomitant drug use will also be monitored in the in-hospital registry.	• Sections 4.4 and 4.5 of the SPC contain language cautioning against concomitant use with these drugs.
Paediatric population	• Continue to analyse paediatric data from clinical trials • Periodically review and analyse safety database for any potential post-marketing use in the paediatric or adolescent population.	• Section 4.2 of SPC states that EFIENT is not recommended for use in children and adolescents due to a lack of data on safety and efficacy.
Pregnant/Lactating women	• Continue to analyse AE reports in clinical trials • Periodically review and analyse safety database for any potential post-marketing use in pregnant or lactating women • Routine pharmacovigilance, targeted surveillance with specific follow-up form for pregnancy and lactation, safety surveillance for safety signal detection associated with these events. Pharmacovigilance, targeted surveillance with specific follow-up form for exposure condition, safety surveillance for safety signal detection associated with the exposure condition.	• Section 4.6 of SPC recommends a risk/benefit evaluation approach with regard to pregnancy, and does not recommend use of EFIENT during breastfeeding.

Summary of the Risk Management Plan for EFIENT (prasugrel) – concluded

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Missing Information (cont.)		
Subjects without clinical manifestation of ACS or with ACS not managed by PCI (requiring immediate CABG or suitable for medical management only)	• Continue to analyse AE reports in clinical trials • Periodically review and analyse safety database for any spontaneously reported case associated with these situations • CAD subjects with no symptom of ACS may be detected by routine pharmacovigilance activities. • Medically-managed ACS subjects not planned to be managed by PCI will be studied in Study TABY.	• Indication statement in Section 4.1 of the SPC includes definition of the targeted population for EFIENT, i.e. ACS *undergoing* PCI.
Subjects with severely compromised cardiac status (cardiogenic shock, Class IV CHF, refractory ventricular arrhythmia)	• Routine pharmacovigilance, safety surveillance for safety signal detection associated to prasugrel use in this specific subpopulation.	• Indication statement in Section 4.1 of the SPC describes the target patient population as follows: o patients with acute coronary syndrome (i.e. unstable angina, non-ST segment elevation myocardial infarction [UA/NSTEMI] or ST segment elevation myocardial infarction [STEMI]) undergoing primary or delayed percutaneous coronary intervention (PCI).
Subjects with severe hepatic impairment.	• Continue to analyse AE reports in clinical trials • Periodically review and analyse safety database for any spontaneously reported case associated with severe hepatic impairment • In –hospital registry will allow for identification of subjects with possible liver damage.	• Contraindication for patients with "severe hepatic impairment (Child Pugh Class C)" in Section 4.3 of the SPC.

The CHMP, having considered the data submitted in the MA application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product:
The MAH should provide educational material to all physicians who may be involved in treating patients with prasugrel. The format and means of dissemination, of this material should be discussed with the appropriate learned societies. The results of the discussion, and where appropriate the material, should be agreed with the national competent authority and be available prior to launch in each member state.

The educational material should include:

- A copy of the SPC
- Emphasis that:

- Severe haemorrhagic events are more frequent in patients ≥ 75 years of age (including fatal events) or those weighing < 60 kg
- Treatment with prasugrel is generally not recommended for patients of ≥ 75 years of age.
- If, after a careful individual benefit/risk evaluation by the prescribing physician, treatment is deemed necessary in the ≥ 75 years age group then following a loading dose of 60 mg, a reduced maintenance dose of 5mg should be prescribed.
- Patients weighing < 60 kg should have a reduced maintenance dose of 5mg
  The evidence for a 5mg dose is based only on PK/PD analyses and no clinical data currently exist on the safety of this dose in the at risk sub groups.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. There are a number of quality issues that will be resolved as Follow-up Measures within an agreed timeframe. None of these issues is expected to have a negative impact on the Benefit Risk balance of the product.

Non-clinical pharmacology and toxicology

The non clinical pharmacological programme provided an adequate characterisation of the pharmacological properties of prasugrel. In *ex vivo* studies with rats, dogs, and cynomolgus monkeys, prasugrel demonstrated dose-dependent inhibition of ADP-induced platelet aggregation. The *in vivo* effects of prasugrel were assessed in nonclinical pathophysiological models of thrombotic challenge. Administration of prasugrel at clinical doses is not expected to produce secondary pharmacology effects related to CNS, cardiovascular, respiratory, renal, or GI function or to have an affect on QT interval. Additive or synergistic platelet inhibitory effects of the co-administration of prasugrel with aspirin have also been demonstrated. The active metabolite of prasugrel R-138727 is chiral; with two most potent enantiomers of R-138727 comprise approximately 84% of the metabolite in human plasma. The overall metabolism of prasugrel was thoroughly investigated.
The primary effects of prasugrel observed during repeat-dose toxicology studies included decreased body weight relative to control in rodents that was occasionally accompanied by decreased food consumption, and increased liver weight and histologic changes in the liver considered to be related to microsomal enzyme induction. Prasugrel did not exhibit genotoxic properties when tested *in vitro* nor *in vivo*. The increase in liver tumours observed in mice dosed with prasugrel is not considered a relevant human risk, and this is adequately reflected in the proposed prescribing information. Prasugrel did not exhibit toxicity towards fertility and early embryonic development and did not show embryo-foetal toxicity. The SPC obtains adequate statements.

Non-clinical and clinical data indicate that evidence of the phototoxic potential of prasugrel is weak and of questionable clinical relevance. Nevertheless, phototoxicity was included as a potential risk in the RMP.

A complete environmental risk assessment has been conducted for prasugrel. No likely risk has been identified for aquatic organisms in either ground water or surface water, or for sediment dwelling organisms.

Efficacy

A single pivotal superiority trial supports the use of prasugrel (60 mg LD and 10 mg MD) in patients with ACS with scheduled PCI. This study demonstrated that treatment with prasugrel, as compared with clopidogrel at the standard approved dose resulted in a statistically significant reduction in the rate of the primary composite efficacy endpoint of cardiovascular death, nonfatal MI, or nonfatal stroke. The reduction of the incidence of primary composite endpoint was primarily driven by a

reduction in the number of cardiac ischemic events, in particular nonfatal MI. These events are not considered to be harmless periprocedural increases in biochemical markers after index PCI alone. Prasugrel treatment might initially protect against smaller myocardial infarctions related to index PCI, however, the absolute reduction of nonfatal myocardial infarction in the all ACS population continued to increase throughout the study period. The absolute percentage reductions were 0.94, 1.57, 1.65, and 2.15 at 3 days, 30 days, 90 days, and at study end, respectively. When similar analyses are done separately in UA/NSTEMI and STEMI populations, continued increases in the absolute reduction of nonfatal myocardial infarction were also observed. This data suggests that prasugrel treatment protects against short-term as well as long-term cardiac ischemic events. A relative risk reduction of about 20% was observed in UA/NSTEMI, STEMI, and all ACS populations. The approximate 2% absolute risk reduction observed in these populations is considered clinically meaningful.

Data from subgroup analyses suggest that patients with a history of diabetes mellitus could benefit from prasugrel treatment. In response to a question on this topic from the CHMP, a general discussion of platelet reactivity and ischemic risk in diabetic patients in the setting of ACS preceded a presentation of clinical outcomes in subjects with diabetes mellitus in study TAAL. Diabetic subjects, not on insulin, had higher relative and absolute risk reduction if randomized to prasugrel, compared to non-diabetic patients randomized to prasugrel. Even higher risk reductions were observed in diabetic patients on insulin, with the absolute risk reduction (primary efficacy endpoint) with prasugrel *vs* clopidogrel being 6.4%. The relative risk reduction was 37% in this sub-population. Qualitatively similar effects of diabetic status were observed for a number of other efficacy endpoints. Diabetic status seems to affect efficacy and safety differentially, favouring prasugrel treatment in diabetic subjects in particular. It is also acknowledged that consistency of the efficacy benefit across pre-specified primary and secondary endpoints supports the notion that the treatment benefit in diabetic subjects is not a chance finding. However, in contrast the clinical data indicate that patients with a history of prior TIA or stroke are harmed by treatment with prasugrel when compared to treatment with clopidogrel. This effect on the primary efficacy endpoint seems to be driven primarily by an increase in new strokes. For this reason, a history of prior stroke or TIA is now listed under contraindications in the SPC.

In the < 60 kg group, a reduced maintenance dose of 5 mg following a 60 mg loading dose should be prescribed. If treatment is deemed necessary in the ≥ 75 years age group, a reduced maintenance dose of 5 mg following a 60 mg loading dose should be prescribed after a careful individual benefit/risk evaluation by the prescribing physician. There are no adequate clinical data to support this recommendation; thus, the positive clinical outcome of this reduced maintenance dose remains to be seen. Further clinical studies to address this issue will be conducted.

Safety

The safety of prasugrel was comparable to clopidogrel with respect to the incidence of AEs, SAEs and deaths, as well as pre-specified, non-hemorrhagic, clinically relevant TEAEs and laboratory values (thrombocytopenia, neutropenia, leucopenia, allergic reactions, including angioedema, abnormal hepatic function, and torsades de pointes/QT prolongation). The overall incidence of study drug discontinuation was higher in the prasugrel group compared to clopidogrel (approximately 1% greater) primarily due to a higher incidence of hemorrhagic AEs.
Adverse events related to haemorrhage occurred with a statistically significantly higher incidence in the prasugrel treated patients compared to clopidogrel and this was consistent in the majority of subgroups of bleeding events.

Recent clinical studies of anti-thrombotic agents have suggested that major bleeding events may predict an increased risk of CV or non-CV death in the early weeks following the bleeding event and the administration of blood units are called to play a special role on this. This increased risk is also evident in study TAAL. Additionally in this regard, there is ongoing controversy about the efficacy and safety of blood transfusions in the ACS context. To the extent that current recommendations indicate that in mild to moderate anaemia (Hct >25% and Hb >8 g/dl) blood transfusions may be related with an increase risk of death at 30 days and should be avoided if haemodynamically well tolerated. As mentioned earlier, in a post hoc analysis in Study TAAL assessment of long-term

outcomes for subjects experiencing a Non-CABG-related TIMI Major bleeding event indicated that, after 30 days from the event, the risk for major adverse CV events was not higher than that observed in subjects not experiencing the bleeding event. Beyond 30 days, mortality was comparable in those patients who did not have a major bleeding and those who had a major bleeding. Likewise, within 30 days, mortality was comparable for prasugrel and clopidogrel treated patients who did not experience a major bleeding. However, after a TIMI major bleeding, mortality (within 30 days) was significantly higher for prasugrel treated patients compared to clopidogrel treated patients for the All ACS population and its clinical presentations UA/NSTEMI and STEMI. When considering only the so-called ideal population (patients without a history of TIA or stroke, subjects ≥75 years of age, or subjects <60 kg), all cause mortality is still numerically higher after TIMI major bleeding in prasugrel compared to clopidogrel but the difference was not statistically significant. The deaths were predominantly attributable to fatal bleedings.

Apart from the direct haemodynamic consequences of the bleeding episode, which is also associated with the risk of ischemic events occurring in relation to bleeding induced hypotension or transfusions, an important component of the risk is the potential need to interrupt the antiplatelet and antithrombotic drugs which can lead to an increase of ischemic events. In clinical practice the risk of interrupting antithrombotic and antiplatelet treatments must be weighed against the risk of a thrombotic event, particularly if the patient has been submitted to revascularization and stent implantation. The SPC states that premature discontinuation of any antiplatelet agent could result in increased risk of ischaemic events. Further, the SPC includes a warning that in patients with active bleeding in whom reversal of pharmacological effects of prasugrel is required, platelet transfusion may be appropriate.

Colon cancer occurred with a higher incidence in patients treated with prasugrel, but this was assumed to be the consequence of a higher rate of detection rate due to bleeding associated with this therapy.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 2.5 adequately addressed these concerns

- User consultation

User testing of the package leaflet was performed using methodology, which methodology follows the readability guideline. No revisions of the PL were made between test rounds. In conclusion, the user testing of this PL version is judged acceptable.

Risk-benefit assessment

Superiority of prasugrel over clopidogrel was shown in the clinical setting when the primary composite efficacy endpoint - CV death, nonfatal MI, or nonfatal stroke at a median follow-up of 14.5 months - is considered, and was primarily driven by a reduction in MIs. Peri-procedural as well as spontaneous MIs were decreased with prasugrel.

The treatment benefit associated with prasugrel was generally preserved across the major pre-specified subgroups. The reduction in ischemic events with prasugrel was evident regardless of the adjunctive therapy or stent type selected during PCI. Clinical data suggest that patients with a history of diabetes could benefit in particular from treatment with prasugrel. Vice versa, although not reaching statistical significance, the data also suggest that subjects with a history of prior TIA or stroke are harmed by treatment with prasugrel compared to treatment with clopidogrel. This patient subgroup is contraindicated.

Key safety issues were primarily related to the risk of bleeding. A statistically higher incidence of hemorrhagic AEs was observed for prasugrel *vs* clopidogrel. These were related to higher rates of GI bleeding, surgical site bleeding, bleeding at other sites not pre-specified or unknown and retroperitoneal bleeding. Fatal hemorrhagic AEs were higher in the prasugrel group. The finding of significantly increased hemorrhagic events in the prasugrel studies was in general seen for the All-

ACS population and the UA/NSTEMI population. In the STEMI subgroup incidences of the different hemorrhagic events were in general numerically higher in the prasugrel group compared to the clopidogrel group, however, the differences were in most cases not statistically significant. Subgroups vulnerable to bleeding were patients ≥75 years old, patients with body weight <60 kg, and patients with a history of prior TIA or stroke. Further analyses indicated that increased mortality after TIMI major bleedings with prasugrel within the first month of treatment appeared to be related mainly to fatal bleedings. The higher bleeding risk associated with the use of prasugrel is appropriately described in the SPC and guidance has been given to minimise the use of prasugrel in populations at higher risk of bleeding.

It should also be remembered that safety in a real clinical setting tends to be worse than observed under controlled conditions. Attention should therefore be paid to the possible risk of ischemic events which may occur in relation to major bleeding events, e.g. by discontinuation of antiplatelet therapy, bleeding induced hypotension, or transfusions.

For patients ≥ 75 years, treatment is generally not recommended. For selected subgroup of patients ≥75 years for whom prasugrel treatment is deemed necessary, a reduced maintenance dose of 5 mg should be prescribed. Patients < 60 kg should receive a reduced maintenance dose of 5 mg. The main limitation of this recommendation for a prasugrel dose adjustment is the current lack of clinical data supporting the safety and efficacy for the treatment of these subgroups with a reduced maintenance dose of 5 mg. Additional clinical studies to assess the 5 mg dose in the relevant at risk populations are necessary and are planned, as discussed earlier.

In summary, the following table describes Number Needed to Treat (NNT) and Number Needed to Harm (NNH) for different subgroups.

NNT (Primary Efficacy Outcome) and NNH (TIMI Major bleedings) - Study TAAL; All randomized Subjects

Subgroup	Efficacy Sample size	NNT (Primary efficacy [1])	NNT (All death, MI, Stroke)	NNH (TIMI Major)	NNH (TIMI Life-Threatening)
All ACS	13608	49	52	195	234
UA/NSTEMI	10074	52	58	163	186
STEMI	3534	42	40	444	888
Gender					
Female	3523	72	59	109	226
Male	10085	44	50	254	229
Age					
≥75 years	1809	102	192	110	98
<75 years	11799	45	47	220	295
Weight					
<60 kg	668	81	58	36	81
≥60 kg	12769	48	52	267	284
Region					
North America	4310	35	36	328	1243
Western EU	3553	73	70	198	222
Eastern EU	3322	61	75	102	163
Medical History					
Diabetics	3146	23	22	795	313
Non-diabetics	10462	74	87	159	218
Prior TIA/Stroke	518	-23	-20	33	32
No prior TIA/Stroke	13090	43	45	243	311
Ideal population[2]	10,804	39	40	312	368

[1] CV death, non-fatal MI, or non-fatal stroke
[2] that is, population for whom a 10 mg MD is recommended

Sufficient evidence has been provided that the timing of clopidogrel LD, which is recommended to be given immediately, and not, as in study TAAL after diagnostic angiography, did not substantially influence the efficacy of prasugrel *vs* clopidogrel.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.

- the following additional risk minimisation activities were required:

The MAH should provide educational material to all physicians who may be involved in treating patients with prasugrel. The format and means of dissemination, of this material should be discussed with the appropriate learned societies. The results of the discussion, and where appropriate the material, should be agreed with the national competent authority and be available prior to launch in each member state.

The educational material should include:

- A copy of the SPC
- Emphasis that:
  - Severe haemorrhagic events are more frequent in patients ≥ 75 years of age (including fatal events) or those weighing < 60 kg
  - Treatment with prasugrel is generally not recommended for patients of ≥ 75 years of age.

- If, after a careful individual benefit/risk evaluation by the prescribing physician, treatment is deemed necessary in the ≥ 75 years age group then following a loading dose of 60 mg, a reduced maintenance dose of 5mg should be prescribed.
- Patients weighing < 60 kg should have a reduced maintenance dose of 5mg The evidence for a 5mg dose is based only on PK/PD analyses and no clinical data currently exist on the safety of this dose in the at risk sub groups.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus decision that the risk-benefit balance of Efient "co-administered with acetylsalicylic acid (ASA), for the prevention of atherothrombotic events in patients with acute coronary syndrome (i.e. unstable angina, non-ST segment elevation myocardial infarction [UA/NSTEMI] or ST segment elevation myocardial infarction [STEMI]) undergoing primary or delayed percutaneous coronary intervention (PCI)" was favourable and therefore recommended the granting of the marketing authorisation.

Exhibit E

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Prasugrel

Stephen D. Wiviott, Elliott M. Antman and Eugene Braunwald

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Prasugrel

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Platelet adhesion, activation, and aggregation play key roles in both normal hemostasis and pathological thrombosis. In the latter, these factors are paramount in the initiation of the intracoronary thromboses that cause acute coronary syndromes (ACS) and the ischemic complications following coronary artery interventions, including recurrent myocardial infarction (MI) and stent thrombosis.[1] The interaction of ADP with purenergic $P2Y_1$ and $P2Y_{12}$ receptors serves to amplify and sustain platelet activation.[2] Activated platelets expose glycoprotein IIb/IIIa receptors, which crosslink with fibrin to form platelet aggregates. These aggregates cause mechanical disruption of flow and may embolize downstream, causing microvasculature obstruction that results in myocardial ischemia and infarction.

The important role of antiplatelet agents in the management and prevention of the complications after ACS and percutaneous coronary intervention (PCI) is related directly to the physiological events noted above.[3–7] Thienopyridine antiplatelet agents interfere with platelet activation and aggregation induced by ADP. There are 3 members of the thienopyridine class of antiplatelet agents currently available for clinical use: ticlopidine, clopidogrel, and the subject of this review, prasugrel. All 3 agents are prodrugs and require conversion to an active metabolite to exhibit an antiplatelet effect (Figure 1). The active metabolite of the thienopyridine binds irreversibly to the $P2Y_{12}$ receptor, blocking the binding of ADP and thereby inhibiting platelet activation and aggregation[8] and leading to the clinical benefits and risks of these agents. The benefits of ticlopidine were shown in a series of trials comparing dual antiplatelet therapy with aspirin plus an oral anticoagulant.[9,10] Ticlopidine is limited by the need to take the drug twice daily, by poor tolerability, notably gastrointestinal distress, but most important by severe side effects, including bone marrow aplasia.[11] Clopidogrel plus aspirin dual antiplatelet therapy has become the standard of care for the support of patients undergoing PCI with stenting largely on the basis of a better tolerability profile.[12,13] Clinical trials established the benefits of clopidogrel across the spectrum of ACS, including unstable angina (UA), non–ST-elevation MI (NSTEMI), and ST-elevation MI (STEMI).[14–16] American College of Cardiology/American Heart Association guidelines recommend dual antiplatelet therapy with aspirin and clopidogrel in patients with ACS for up to 1 year regardless of syndrome type or treatment strategy (medical, PCI, or surgery).[6]

Pharmacological Limitations of Clopidogrel

Despite the major benefits of clopidogrel alone and in combination with aspirin for patients with ACS and for those undergoing PCI, important pharmacological limitations are associated with its use.[17] The antiplatelet effects of clopidogrel have a delayed onset (several hours after ingestion), and there is substantial variability in response among patients. A growing number of studies have linked poor antiplatelet response to clopidogrel to adverse clinical outcomes, particularly coronary ischemia and stent thrombosis.[18–21] From these limitations, the evaluation of more intensive and consistent antiplatelet therapy compared with clopidogrel has been fostered. One such agent, prasugrel, a third-generation thienopyridine, is the focus of this review.

Pharmacology of Prasugrel

Prasugrel requires enzymatic metabolism to exert its antiplatelet effects (Figure 1).[22] The parent molecule, prasugrel, is rapidly hydrolyzed by esterases, such as those located in the intestine and blood, to a thiolactone intermediate metabolite R-95913. The parent molecule is therefore not detectable in the plasma. This intermediate metabolite undergoes subsequent activation by a single cytochrome P450 (CYP)–dependent step (predominantly CYP3A and CYP2B6) to form the sulfhydryl-containing active metabolite R-138727. This active metabolite binds irreversibly to the platelet $P2Y_{12}$ receptor by covalent linkage of a sulfhydryl group to inhibit platelet activation and aggregation. Prasugrel metabolism differs from clopidogrel metabolism in that the initial hydrolysis of the parent clopidogrel compound results in inactivation of a substantial fraction (≈85%) of the absorbed drug, and the subsequent activation requires 2 CYP-dependent steps (Figure 1).[22] The CYP enzymes involved in the metabolism of clopidogrel and prasugrel are polymorphic, differing between individuals, and are responsible in part for the interpatient variability of clopidogrel response.[23,24] The prasugrel active metabolite concentration peaks in the plasma at ≈30 minutes, and the concentration is proportional to a dose between 5 and 60 mg. When not bound to platelets, the active metabolite of prasugrel has an elimination half-life of ≈7 hours.[25] Prasugrel does

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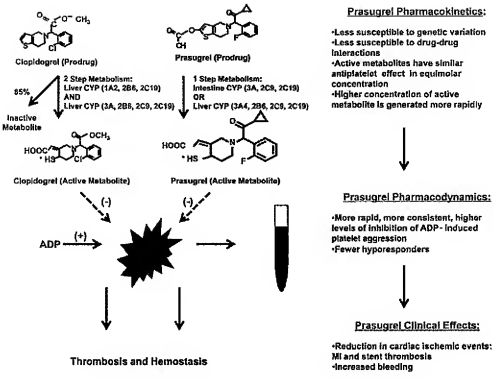

Figure 1. Schematic representation of the relationship between thienopyridine metabolism, pharmacological effects, and clinical outcomes.

not have clinically relevant interactions with inducers or inhibitors of the cytochrome P450 system.[26] The active metabolite concentration and pharmacodynamic response of prasugrel were not affected by moderate renal impairment compared with healthy subjects.[27] In patients with end-stage renal disease, active metabolite concentrations were ≈40% lower, but similar platelet inhibition was noted. In patients with moderate liver disease, no effects on prasugrel pharmacokinetics or pharmacodynamics have been observed. Prasugrel has not been tested in severe hepatic disease.[28]

In clinical pharmacology studies of healthy subjects, no effect of age on prasugrel pharmacokinetics or pharmacodynamics was observed with age between 20 and 80 years.[29] In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel Thrombolysis in Myocardial Infarction 38 (TRITON–TIMI 38), however, patients ≥75 years had 19% higher exposure to the active metabolite of prasugrel compared with those <75 years of age and 25% higher exposure compared with patients <60 years of age. In both clinical pharmacology studies and TRITON–TIMI 38, prasugrel pharmacokinetics were affected by body weight, with higher exposure in subjects with lower body weight. In TRITON–TIMI 38, patients <60 kg had 30% higher exposure than patients ≥60 kg and 42% higher exposure than patients ≥85 kg. In the analysis of TRITON–TIMI 38, pharmacokinetics were not appreciably affected by diabetes, smoking, or renal impairment.[30]

In equimolar concentrations, the active metabolites of prasugrel and clopidogrel have similar antiplatelet effects.[31] Therefore, the more rapid and consistent conversion of prasugrel from the inactive prodrug to its active metabolite and the ability of patients to generate higher concentrations of the active metabolite provide the mechanistic basis for the differences in the pharmacological profiles of the 2 drugs.[32] A 2-phase crossover study comparing loading doses of prasugrel (60 mg) and clopidogrel (300 mg) in healthy subjects showed higher maximal levels and less variable inhibition of ADP-induced platelet aggregation with prasugrel (79±9%) than with clopidogrel (35±25%).[32] A difference in platelet inhibition was apparent between the 2 drugs by 30 minutes and persisted to 24 hours, the duration of measurement. This same study also demonstrated several patients with low-level (<20% induced platelet aggregation) response to clopidogrel but no such patients with prasugrel. These observations resulted from the ≈10-fold higher levels of prasugrel active metabolite than clopidogrel active metabolite.[32]

Many physicians in clinical practice use higher doses of clopidogrel than the standard approved dose. This practice is supported in part by clinical practice guidelines,[12] small clinical outcomes studies, observational studies, and meta-analyses.[33–35] The Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Interventions (CURRENT OASIS 7) trial[36] compared high-loading- and -maintenance-dose clopidogrel (600-mg loading dose followed by 150 mg/d for 7 days) with standard dosing (300-mg loading dose followed by 75 mg/d) in ≈25 000 patients with ACS treated with or without coronary angiography. The trial did not meet its primary end point of a reduction of cardiovascular death, MI, or stroke in the overall cohort of ACS patients, and more CURRENT major bleeding was observed with the higher-dose clopidogrel strategy. Lower ischemic event rates were observed with the combination of high-dose clopidogrel and high-dose aspirin (300 to

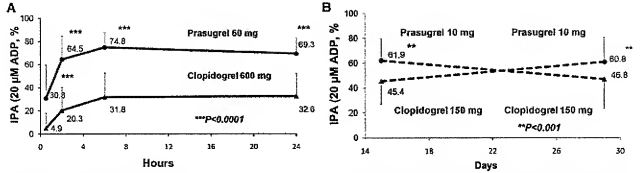

Figure 2. The main results of PRINCIPLE–TIMI 44. Prasugrel 60 mg resulted in higher levels of platelet inhibition than 600 mg clopidogrel in the acute phase, and 10 mg prasugrel resulted in higher levels of platelet inhibition than 150 mg clopidogrel in the chronic phase. IPA indicates inhibition of platelet aggregation. Data derived from Wiviott et al.[37]

325 mg daily). In a postrandomization subgroup of patients who underwent PCI, there was a reduction of ischemic events, including MI and stent thrombosis, with higher-dose clopidogrel compared with the standard dose.[36]

The Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation (PRINCIPLE)–TIMI 44 trial compared prasugrel and clopidogrel using the CURRENT OASIS 7 clopidogrel dose regimen (600-mg loading dose followed by 150 mg daily). This was a 2-phase crossover study in patients with coronary artery disease undergoing cardiac catheterization with planned PCI. Prasugrel 60-mg loading dose showed higher levels of platelet inhibition than 600 mg clopidogrel, and 10 mg prasugrel showed greater levels of platelet inhibition than 150 mg clopidogrel daily[37] (Figure 2). A significant difference in induced platelet aggregation emerged as soon as 30 minutes and persisted throughout the first 24 hours. In fact, by 30 minutes, the levels of inhibition with prasugrel 60 mg were similar to the maximal inhibition achieved with clopidogrel 600-mg loading dose (Figure 2A). In a crossover design, higher levels of induced platelet aggregation were also observed with 10 mg prasugrel compared with 150 mg clopidogrel after 2 weeks of therapy (Figure 2B).

Clinical Outcomes Trials of Prasugrel

The Joint Utilization of Medications to Block Platelets Optimally (JUMBO)–TIMI 26[38] trial was a phase II, dose-ranging, safety study of prasugrel compared with clopidogrel in patients undergoing PCI. In this study, 904 subjects were randomized to 1 of 3 prasugrel dosing strategies with 2 prasugrel loading doses and 3 prasugrel maintenance doses (40/7.5 mg, 60/10 mg, and 60/15 mg) compared with clopidogrel (300/75 mg) and followed up for 30 days. Bleeding rates overall were low, and there were no statistically significant differences among treatment groups for the primary safety end point of TIMI major or minor bleeding. However, numerically more patients had bleeding in the combined prasugrel group (hazard ratio [HR], 1.42; 95% confidence interval [CI] 0.40 to 5.08) compared with clopidogrel, and the 60/15 mg prasugrel arm tended to have higher rates of TIMI minimal bleeding. The study was not powered for efficacy end points, and a nonsignificant difference (HR, 0.76; 95% CI, 0.46-to 1.24) was observed for the primary end point of major adverse cardiovascular events in favor of prasugrel-treated patients compared with clopidogrel-treated patients. The combination of clinical data from JUMBO–TIMI 26 and platelet function data in healthy volunteers[32] and patients[39] served as the basis for dose selection for the pivotal phase II, registration pathway trial of prasugrel.

Efficacy

The majority of clinical outcomes data for prasugrel comes from the phase III TRITON–TIMI 38 trial. In this trial, 13 608 subjects with moderate- to high-risk ACS, including UA, NSTEMI, and STEMI with planned PCI, were randomized to receive either clopidogrel 300-mg loading dose followed by 75 mg daily or prasugrel 60-mg loading dose followed by 10 mg daily. Subjects with UA/NSTEMI or STEMI treated initially with medical therapy could be randomized and treated only after the coronary anatomy was known to be suitable for PCI; subjects with STEMI and planned primary PCI could be randomized and treated on first contact. Subjects with recent clopidogrel use, known bleeding diathesis, or other high-risk features for bleeding were excluded.[40] Subjects were treated for a median of 14.5 months.

The primary end point was the composite of death from cardiovascular causes, nonfatal MI, or nonfatal stroke. Subjects randomized to prasugrel had fewer primary end-point events (9.9% versus 12.1%; HR, 0.81; 95% CI, 0.73 to 0.90; $P<0.001$) compared with clopidogrel, as shown in Figure 3A and Table 1.[41]

The primary efficacy end point (Table 1) was driven by a 24% reduction in MI including both fatal and nonfatal MI. Cardiovascular death and total mortality were numerically but not statistically lower (total mortality, 3.0% versus 3.2%; HR, 0.95; 95% CI, 0.78 to 1.16; in STEMI, 3.3% versus 4.3%; HR, 0.76; 95% CI, 0.54 to 1.07; in UA/NSTEMI, 2.9% versus 2.8%; HR, 1.08; 95% CI, 0.84 to 1.38), and no effect was seen on total stroke. Additional analyses of reduction in MI using the American College of Cardiology/American Heart Association/European Society of Cardiology universal MI definition showed similar reductions in procedural MI and spontaneous MI, small and large MIs (based on peak biomarkers), investigator reported MIs, and MIs both early (within 30 days) and after 30 days.[42] The reduction in clinical ischemic events was also notable for a reduction in urgent target vessel revascularization (2.5% versus 3.7%; HR, 0.66;

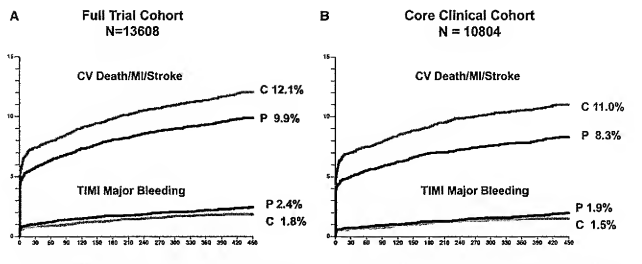

Figure 3. A, TRITON–TIMI 38 main results in the overall cohort. B, Main results figure in core clinical cohort (no history of stroke/transient ischemic attack, age <75 years, and weight ≥60 kg). CV indicates cardiovascular; C, clopidogrel; P, prasugrel; NNT, number needed to treat; NNH, number needed to harm. Data derived from Wiviott et al.[41]

95% CI, 0.54 to 0.81; $P<0.001$). A key finding from TRITON–TIMI 38 was a marked reduction in stent thrombosis among patients receiving prasugrel. Overall for the duration of the trial, Academic Research Consortium–defined definite or probable stent thrombosis was reduced by 52% (1.1% versus 2.4%; HR, 0.48; 95% CI, 0.36 to 0.64; $P<0.001$) and definite (angiographic or autopsy proven) stent thrombosis by 58% (0.9% versus 2.0%; HR, 0.42; 95% CI, 0.31 to 0.59; $P<0.001$; Figure 4A)[43] in patients who received prasugrel. These findings were similar whether patients received bare metal stents or drug-eluting stents. The reduction in stent thrombosis was also noted both early, ie, within 30 days after randomization (0.64% versus 1.56%; HR, 0.41; 95% CI, 0.29 to 0.59; $P<0.0001$), and after 30 days (0.49% versus 0.82%; HR, 0.60; 95% CI, 0.37 to 0.97; $P=0.03$; Figure 4B).

Safety

Consistent with the more potent antiplatelet effects observed with prasugrel in TRITON–TIMI 38, higher rates of bleeding were observed. The key safety end point of non–coronary artery bypass graft (CABG)–related TIMI major bleeding was observed more frequently with prasugrel (2.4% versus 1.8%; HR, 1.32; 95% CI, 1.03 to 1.68; $P=0.03$; Figure 3A).[41] In addition to the key safety end point of TIMI major bleeding, there was an excess of non-CABG–related TIMI major or minor bleeding (5.0% versus 3.8%; HR, 1.31; 95% CI, 1.11 to 1.56; $P=0.002$) and bleeding requiring transfusion (4.0% versus 3.0%; HR, 1.34; 95% CI, 1.11 to 1.63; $P<0.001$). Among non-CABG–related bleeding, the excess was driven predominantly by an increase in spontaneous bleeding (1.6% versus 1.1%; HR, 1.51; 95% CI, 1.09 to 2.08; $P=0.01$). Instrumented bleeding and bleeding related to trauma were less frequent in both groups, and rates

Table 1. Key Efficacy and Safety Results From TRITON–TIMI 38

End Point	Clopidogrel, %	Prasugrel, %	Absolute Risk Difference, %	HR	95% CI	P
CVD/MI/CVA	12.1	9.9	2.2	0.81	0.73–0.90	<0.001
MI	9.7	7.4	2.3	0.76	0.67–0.85	<0.001
Urgent TVR	3.7	2.5	1.2	0.66	0.54–0.81	<0.001
TIMI major bleeding*	1.8	2.4	0.6	1.32	1.03–1.68	0.03
TIMI major or minor bleeding*	3.8	5.0	1.2	1.31	1.11–1.56	0.002
Bleeding requiring transfusion*	3.0	4.0	1.0	1.34	1.11–1.63	<0.001
All-cause death, MI, stroke, TIMI major bleeding*	13.9	12.2	1.7	0.87	0.79–0.95	0.004

CVD indicates cardiovascular death; CVA, cerebrovascular accident (stroke); and TVR, target vessel revascularization.
*Not related to coronary artery bypass surgery.
Data derived from Wiviott et al.[41]

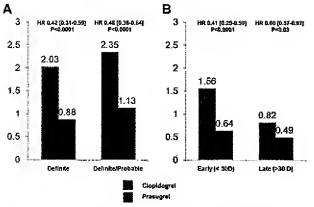

Figure 4. Stent thrombosis in TRITON–TIMI 38. A, Stent thrombosis in the overall cohort for the duration of the trial. B, Academic Research Consortium definite or probable stent thrombosis by timing. Data derived from Wiviott et al.[43]

were similar between the 2 treatment arms. Among the non-CABG major bleeds, there was an excess of life-threatening bleeding and, though rare, fatal bleeding. Intracranial bleeding was not increased among the patients treated with prasugrel (0.3% of both treatment arms).

The relative bleeding excess with prasugrel tended to be similar among major subgroups and tended to continue to accumulate over time; no significant difference in TIMI major bleeding was observed by 30 days (1.03% versus 0.87%; HR, 1.19; 95% CI, 0.84 to 1.68; $P=0.34$), and bleeding in this time period was most often procedure related with both agents. However, after 30 days, a significant excess in TIMI major bleeding was observed (1.42% versus 0.97%; HR, 1.48; 95% CI, 1.04 to 2.09; $P=0.03$) and was more commonly spontaneous bleeding, particularly gastrointestinal bleeding. No significant interaction between time of follow-up and treatment was observed. Because TRITON–TIMI 38 was designed as a PCI trial in which coronary anatomy had to be known to be suitable for PCI before randomization, CABG was infrequent. TIMI major bleeding was identified in 13% of prasugrel-treated patients who underwent CABG (0.4% of the total cohort) compared with 3% of clopidogrel-treated patients who underwent CABG (0.1% of the total cohort; HR, 4.73; 95% CI, 1.90 to 11.82; $P<0.001$).

To assess the balance between safety and efficacy, a prespecified net outcome end point of all-cause death, MI, stroke, and non-CABG TIMI major bleeding was evaluated in TRITON–TIMI 38. In the overall cohort, this end point favored prasugrel (12.2% versus 13.9%; HR, 0.87; 95% CI, 0.79 to 0.95; $P=0.004$). This net outcome was robust to sensitivity analyses, including less severe bleeding episodes (such as TIMI minor bleeding).[44] In addition to the prespecified safety end points, a careful assessment of reported adverse events identified a slight excess in patients with new or worsened malignancies, particularly colon cancers, which may have resulted from ascertainment bias caused by earlier identification in the prasugrel group as a result of evaluations for bleeding or anemia. The US Food and Drug Administration determined that there was not sufficient biological evidence to suggest that prasugrel was a carcinogen or a tumor promoter and requested additional studies to assess the potential cancer risk.[45]

Subgroups

Several subgroups of patients in TRITON–TIMI 38 are of both scientific and clinical interest. Three groups were highlighted as being of particular concern: those with previously known stroke, elderly patients, and patients with low body weight. Patients with a self-reported or known history of stroke or transient ischemic attacks (n=518) before enrollment in TRITON–TIMI 38 had a higher rate of primary efficacy events (19.1% versus 14.4%; HR, 1.37; $P=0.15$) driven by an increase in stroke, which differed significantly from the non-stroke cohort (P for interaction=0.02). Coupled with a high rate of bleeding, including more intracranial hemorrhage in this subgroup, the net outcome significantly favored clopidogrel (23.0% versus 16.0%; HR, 1.54; $P=0.04$).

In patients ≥75 years of age (n=1809), a smaller (but directionally consistent) relative reduction in primary efficacy events (17.2% versus 18.3%; HR, 0.94; $P=0.60$), coupled with higher absolute TIMI major bleeding rates (4.2% versus 3.4%; HR, 1.36; $P=0.24$), resulted in a balance between efficacy and safety and a neutral net outcome (21.7% versus 21.5%; HR, 0.99; $P=0.92$). Of particular concern in the elderly patients was the higher rate of spontaneous fatal hemorrhage compared with younger patients. In patients ≥75 years of age, 9 spontaneous fatal hemorrhages were observed with prasugrel and 0 with clopidogrel. In patients <75 years of age, 5 fatal spontaneous hemorrhages were observed with prasugrel and 4 with clopidogrel.

Patients with low body weight (<60 kg; n=668), in whom pharmacokinetic modeling demonstrated an overexposure to the prasugrel active metabolite,[30,41] had an efficacy similar to that of the overall cohort (10.5% versus 12.6%; HR, 0.86; $P=0.54$), had higher rates of bleeding (6.0% versus 3.5%; HR, 1.90; $P=0.09$), and had a neutral net outcome (15.9% versus 15.7%; HR, 1.03; $P=0.89$). It should be recognized that the relative efficacy and safety of prasugrel appear to be optimized in TRITON–TIMI 38 in patients <75 years of age and weighing >60 kg, but it is also likely that the general principle holds that this balance worsens with advancing age and decreasing body weight beyond these precise thresholds (Tables I and II in the online-only Data Supplement).

In contrast, patients with diabetes mellitus had a greater reduction in cardiovascular death/MI/stroke (12.2% versus 17.0%; HR, 0.70; $P<0.001$) than those without diabetes mellitus (9.2% versus 10.6%; HR, 0.86; $P=0.02$; P for interaction=0.09) without an excess in TIMI major bleeding, resulting in a greater improvement in the net outcome.[46] There were consistent benefits among patients presenting with STEMI[47] and those with UA/NSTEMI, although there appeared to be less bleeding excess in patients with STEMI. There were consistent benefits of prasugrel compared with clopidogrel among patients who received varying doses of aspirin,[48] patients with or without glycoprotein IIb/IIIa inhibitors,[49] and those with or without proton pump inhibitors.[50]

A pharmacokinetic substudy of TRITON–TIMI 38 demonstrated that patient groups who had less favorable outcomes, patients who were either <60 kg or ≥75 years of age,

had higher levels of the active metabolite of the drug than did subjects without those characteristics.[30] Modeling data suggest that decreasing the maintenance dose of prasugrel to 5 mg in these subjects would reduce exposure to the active metabolite to levels consistent with those observed in the subjects <75 years of age and ≥60 kg. The efficacy and safety of the 5-mg doses in this population have not been established in a clinical trial but are being tested in the ongoing Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes (TRILOGY ACS) NCT00699998 trial, which is also testing the efficacy of prasugrel compared with clopidogrel in patients with ACS without intended PCI.

Clinical Genetics

As noted above, for clopidogrel or prasugrel to exert an antiplatelet effect, metabolism from an inactive parent compound to the active metabolite that interacts with the $P2Y_{12}$ receptor is required (Figure 1). The CYP enzymes involved in these conversions are known to be subject to common genetic variation resulting in differential function. The combination of the need for metabolism for action and the interpatient variability in metabolic efficiency sets the stage for a pharmacogenomic treatment interaction with clopidogrel. Indeed, several studies have reported that patients who are carriers of a reduced-function allele of *CYP 2C19* are at increased risk of recurrent cardiovascular events, including recurrent MI and stent thrombosis, while being treated with clopidogrel.[24,51–53] In the genetic analysis of TRITON–TIMI 38, ≈30% of subjects tested were carriers of at least 1 common reduced-function allele for *CYP 2C19*. Among the clopidogrel subjects, carriers had an excess of cardiovascular ischemic events, including a 3-fold higher rate of stent thrombosis.[24] These data were supported by consistent effects of the reduced function alleles of *CYP 2C19* showing less generation of active metabolite and less inhibition of platelets in clopidogrel-treated patients.[24] In support of the mechanistic importance of the CYP 2C19 enzyme, a genome-wide association study identified loci associated with genes encoding CYP 2C19 associated with reduced antiplatelet effect of clopidogrel.[54] In contrast to the consistent observations of pharmacogenomic effects on clopidogrel, none of the common variants in the CYP genes tested showed consistent reductions in prasugrel active metabolite generation and the antiplatelet effects of prasugrel.[23] Consequently, subjects assigned to prasugrel in the TRITON–TIMI 38 genetic analysis had no difference in the rates of cardiovascular ischemic events by genotype, suggesting that the more efficient metabolism of prasugrel may render patients less susceptible to such genetic variation.[23]

Regulatory Action and Anticipated Clinical Use

Largely on the basis of the aforementioned TRITON–TIMI 38 trial, prasugrel received approval by both the US Food and Drug Administration and the European Medicines Agency for use in patients with ACS (including STEMI and UA/NSTEMI) undergoing planned PCI (Table 2). Both agencies provided warnings of the bleeding risk with prasugrel, including a "black box" warning by the Food and Drug Administration, and provided contraindications for its use in patients with prior stroke or transient ischemic attack. On the basis of regulatory action, the core clinical cohort recommended for treatment with prasugrel at the studied doses (60 mg and 10 mg) included patients without prior stroke or transient ischemic attack who were <75 years of age and weighed ≥60 kg. This group of patients, who represented 79.4% of the TRITON–TIMI 38 population, exhibited a greater benefit of prasugrel on ischemic end points and had less absolute bleeding difference (Figure 3B). The American and European regulatory agencies approved both 10- and 5-mg tablets of prasugrel and recommended the 5-mg tablet for patients weighing <60 kg (132 lb). For patients ≥75 of age, the US Food and Drug Administration indicated that prasugrel is generally not recommended, but its use may be considered in patients at high risk for recurrent ischemic events such as those with diabetes mellitus or prior MI; it also recommended that when prasugrel is used in the elderly weighing ≥60 kg, prasugrel should be used with its standard dosing regimen. The European Medicines Agency took a slightly different approach, also recommending that prasugrel should generally be avoided in the elderly, but if used, the dose should be lowered to 5 mg.

Summary and Recommendations

Prasugrel is a third-generation thienopyridine with more consistent and efficient metabolism than clopidogrel, the current standard of care. Pharmacodynamic studies have shown that patients taking prasugrel compared with standard or higher doses of clopidogrel have higher levels of thienopyridine active metabolite and higher and more consistent levels of platelet inhibition. Prasugrel appears less susceptible to genetic variation and drug-drug interactions, which can limit the antiplatelet activity and clinical effectiveness of clopidogrel. This pharmacokinetic and pharmacodynamic superiority is translated into improved ischemic outcomes but more bleeding in patients with ACS and planned PCI. These data serve as the first to definitively demonstrate that an agent (or dose of an agent) with higher and more consistent levels of platelet $P2Y_{12}$ inhibition can reduce ischemic events, a key research question in cardiology, and set the stage for further research on personalized medicine, alternative agents, and alternative platelet targets. These data, however, do not indicate the presence of specific goal levels of platelet inhibition for individual patients. Ticagrelor, a direct, nonthienopyridine $P2Y_{12}$ inhibitor, has also been shown to improve clinical outcomes, including total mortality, in patients with ACS compared with clopidogrel,[55] further confirmation of the importance of oral platelet inhibition.

From a patient care standpoint, the favorable balance of risk and benefit in a large majority of the patients studied in TRITON–TIMI 38 served as the basis for approval of prasugrel worldwide by regulatory agencies. Prasugrel is now available for prescription by physicians in the United States and in many countries around the world as an alternative to clopidogrel. Available evidence supports the use of prasugrel either in patients with ACS who are undergoing planned primary PCI for STEMI or in patients with UA/NSTEMI or

Table 2. Summary of Regulatory Action Related to Prasugrel

	US Food and Drug Administration	European Medicines Agency
Indication	Reduction of thrombotic cardiovascular events (including stent thrombosis) in patients with ACS who are to be managed with PCI	Prevention of atherothrombotic events in patients with ACS undergoing primary or delayed PCI
Dose and administration	Initiate treatment with a single 60-mg oral loading dose and continue at 10 mg once daily with or without food	Should be initiated with a single 60-mg loading dose and then continued at 10 mg once a day
		Premature discontinuation of any antiplatelet agent, including prasugrel, could result in an increased risk of thrombosis, MI, or death resulting from the patient's underlying disease
		Treatment of up to 12 mo is recommended unless the discontinuation of prasugrel is clinically indicated
Contraindications	Active pathological bleeding	Hypersensitivity to the active substance or to any of the excipients
	Prior stroke or transient ischemic attack	Active pathological bleeding
		History of stroke or transient ischemic attack
		Severe hepatic impairment (Child Pugh class C)
Warnings and precautions	Can cause significant, sometimes fatal, bleeding Increased risk in patients likely to undergo CABG Premature discontinuation increases the risk of stent thrombosis, MI, and death	Patients with ACS undergoing PCI treated with prasugrel and ASA showed an increased risk of major and minor bleeding; therefore, the use of prasugrel in patients at increased risk of bleeding should be considered only when the benefits in terms of prevention of ischemic events are deemed to outweigh the risk of serious bleedings
Age ≥75 y	Generally not recommended except in high-risk patients (diabetes mellitus or prior MI) in whom its effect appears to be greater and its use may be considered	Generally not recommended and should be undertaken only with caution after a careful individual benefit/risk evaluation by the prescribing physician
		If prescribed, a lower maintenance dose of 5 mg should be used; the 10-mg maintenance dose is not recommended
Weight <60 kg	Consider 5 mg once daily	A 5-mg maintenance dose should be used
Genetics	No relevant effect of genetic variation	No relevant effect of genetic variation
Drug-drug interactions	No relevant effects of inducers or inhibitors of CYP enzymes or drugs that interfere with gastric pH	Can be administered concomitantly with drugs that inhibit or induce CYP enzymes and drugs that interfere with gastric pH

ASA indicates acetylsalicylic acid.

STEMI in whom the coronary anatomy is known to be suitable for PCI who have not been treated with clopidogrel.

Prasugrel has not been compared with pretreatment with clopidogrel. In patients with adequate time for clopidogrel pretreatment before catheterization, the relative efficacy of the 2 strategies of treatment is unknown. Because of its rapid onset of action, loading of prasugrel at the time of angiography can be expected to provide high-level platelet inhibition to support PCI and to reduce the risk of early ischemic events such as acute stent thrombosis. Although CABG-related bleeding was more frequent with prasugrel than with clopidogrel, the ability to use prasugrel to support PCI when coronary anatomy is known should reduce the frequency with which patients who are identified as needing CABG will receive a thienopyridine. Therefore, the use of prasugrel appears particularly well suited for patients who will have cardiac catheterization and PCI within hours (rather than days) of the decision to use a thienopyridine and in whom this more rapid agent can be given peri-PCI and will be active at the time of PCI. Compared with standard-dose clopidogrel, prasugrel provides greater protection from ischemic events, particularly stent thrombosis, repeat revascularization, and MI, throughout 15 months in patients with PCI but with more bleeding. The use of prasugrel appears to have a particularly favorable risk-to-benefit profile in patients with demographic factors that place them at very high risk for recurrent ischemic events but not at high risk for bleeding such as those with ACS and diabetes mellitus and those with STEMI, although clinical trial data do not suggest the limitation of use to these patients.

It may seem logical to extrapolate from previous data to suggest that one may guide therapy on the basis of genetic testing (CYP 2C19 polymorphisms) or to reserve a more potent agent such as prasugrel for patients who are demonstrated to have poor platelet response to clopidogrel on functional testing. However, we do not believe there is currently sufficient evidence or practical availability of rapid testing to identify patients with these particular risk factors. In the set of patients for whom prasugrel is indicated, if physicians wait for the results of genetic testing or initiate clopidogrel and await platelet function testing, the early beneficial effects of rapid and higher level $P2Y_{12}$ inhibition could be lost.

Therefore, as with the incorporation of other new therapies into clinical practice, prasugrel should be used as one component of a treatment strategy to optimize patient outcomes,

balancing ischemic protection, bleeding risk, and cost. From a demographic standpoint, the balance appears to favor efficacy with prasugrel as studied with the exclusion of a well-defined subgroups of patients, including those with prior stroke or transient ischemic attack, the elderly, or patients of low body weight, without additional risk features for recurrent ischemia and strongly favors efficacy in the setting of diabetes mellitus or STEMI. For individual patients, the clinician must integrate the choice and dose of thienopyridine, invasive strategies, vascular access management, stent types, choice of and dose of additional antiplatelet agents, antithrombins, and adjunctive pharmacotherapies.

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References

1. Jennings LK. Mechanisms of platelet activation: need for new strategies to protect against platelet-mediated atherothrombosis. *Thromb Haemost*. 2009;102:248–257.
2. Storey F. The P2Y12 receptor as a therapeutic target in cardiovascular disease. *Platelets*. 2001;12:197–209.
3. Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, Hochman JS, Krumholz HM, Kushner FG, Lamas GA, Mullany CJ, Ornato JP, Pearle DL, Sloan MA, Smith SC Jr, Alpert JS, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Gregoratos G, Halperin JL, Hiratzka LF, Hunt SA, Jacobs AK. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of Patients With Acute Myocardial Infarction). *Circulation*. 2004;110:e82–e292.
4. Antman EM, Hand M, Armstrong PW, Bates ER, Green LA, Halasyamani LK, Hochman JS, Krumholz HM, Lamas GA, Mullany CJ, Pearle DL, Sloan MA, Smith SC Jr, Anbe DT, Kushner FG, Ornato JP, Jacobs AK, Adams CD, Anderson JL, Buller CE, Creager MA, Ettinger SM, Halperin JL, Hunt SA, Lytle BW, Nishimura R, Page RL, Riegel B, Tarkington LG, Yancy CW. 2007 Focused update of the ACC/AHA 2004 guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the Canadian Cardiovascular Society endorsed by the American Academy of Family Physicians: 2007 Writing Group to Review New Evidence and Update the ACC/AHA 2004 Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction, writing on behalf of the 2004 writing committee. *Circulation*. 2008;117:296–329.
5. Smith SC Jr, Feldman TE, Hirshfeld JW Jr, Jacobs AK, Kern MJ, King SB III, Morrison DA, O'Neil WW, Schaff HV, Whitlow PL, Williams DO, Antman EM, Adams CD, Anderson JL, Faxon DP, Fuster V, Halperin JL, Hiratzka LF, Hunt SA, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/SCAI Writing Committee to Update 2001 Guidelines for Percutaneous Coronary Intervention). *Circulation*. 2006;113:e166–e286.
6. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith EE III, Steward DE, Theroux P, Gibbons RJ, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Hiratzka LF, Jacobs AK, Smith SC Jr. ACC/AHA guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction—2002: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). *Circulation*. 2002;106:1893–1900.
7. Silber S, Albertsson P, Aviles FF, Camici PG, Colombo A, Hamm C, Jorgensen E, Marco J, Nordrehaug JE, Ruzyllo W, Urban P, Stone GW, Wijns W. Guidelines for percutaneous coronary interventions: the Task Force for Percutaneous Coronary Interventions of the European Society of Cardiology. *Eur Heart J*. 2005;26:804–847.
8. Herbert JM, Savi P. P2Y12, a new platelet ADP receptor, target of clopidogrel. *Semin Vasc Med*. 2003;3:113–122.
9. Bertrand ME, Legrand V, Boland J, Fleck E, Bonnier J, Emmanuelson H, Vrolix M, Missault L, Chierchia S, Casaccia M, Niccoli L, Oto A, White C, Webb-Peploe M, Van Belle E, McFadden EP. Randomized multicenter comparison of conventional anticoagulation versus antiplatelet therapy in unplanned and elective coronary stenting: the Full Anticoagulation Versus Aspirin and Ticlopidine (FANTASTIC) study. *Circulation*. 1998;98:1597–1603.
10. Leon MB, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, Giambartolomei A, Diver DJ, Lasorda DM, Williams DO, Pocock SJ, Kuntz RE. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting: Stent Anticoagulation Restenosis Study Investigators. *N Engl J Med*. 1998;339:1665–1671.
11. Ekins M, Reichman N, Flatau E. Bone marrow aplasia associated with ticlopidine therapy. *Am J Hematol*. 1993;44:289–290.
12. Smith SC Jr, Feldman TE, Hirshfeld JW Jr, Jacobs AK, Kern MJ, King SB III, Morrison DA, O'Neill WW, Schaff HV, Whitlow PL, Williams DO, Antman EM, Adams CD, Anderson JL, Faxon DP, Fuster V, Halperin JL, Hiratzka LF, Hunt SA, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention—summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/SCAI Writing Committee to Update the 2001 Guidelines for Percutaneous Coronary Intervention). *Catheter Cardiovasc Interv*. 2006;67:87–112.
13. Bertrand ME, Rupprecht HJ, Urban P, Gershlick AH, Investigators FT. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the Clopidogrel Aspirin Stent International Cooperative Study (CLASSICS). *Circulation*. 2000;102:624–629.
14. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, Collins R, Liu LS. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet*. 2005;366:1607–1621.
15. Sabatine MS, Cannon CP, Gibson CM, Lopez-Sendon JL, Montalescot G, Theroux P, Claeys MJ, Cools F, Hill KA, Skene AM, McCabe CH, Braunwald E. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med*. 2005;352:1179–1189.
16. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001;345:494–502.

17. O'Donoghue M, Wiviott SD. Clopidogrel response variability and future therapies: clopidogrel: does one size fit all? *Circulation*. 2006;114: e600–e606.
18. Buonamici P, Marcucci R, Migliorini A, Gensini GF, Santini A, Paniccia R, Moschi G, Gori AM, Abbate R, Antoniucci D. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. *J Am Coll Cardiol*. 2007;49:2312–2317.
19. Gurbel PA, Bliden KP, Samara W, Yoho JA, Hayes K, Fissha MZ, Tantry US. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. *J Am Coll Cardiol*. 2005;46: 1827–1832.
20. Hochholzer W, Trenk D, Bestehorn HP, Fischer B, Valina CM, Ferenc M, Gick M, Caputo A, Buttner HJ, Neumann FJ. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol*. 2006;48:1742–1750.
21. Wenaweser P, Doerffler-Melly J, Imboden K, Windecker S, Togni M, Meier B, Haeberli A, Hess OM. Stent thrombosis is associated with an impaired response to antiplatelet therapy. *J Am Coll Cardiol*. 2005;45: 1748–1752.
22. Jakubowski JA, Winters KJ, Naganuma H, Wallentin L. Prasugrel: a novel thienopyridine antiplatelet agent: a review of preclinical and clinical studies and the mechanistic basis for its distinct antiplatelet profile. *Cardiovasc Drug Rev*. 2007;25:357–374.
23. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias WL, Braunwald E, Sabatine MS. Cytochrome P450 genetic polymorphisms and the response to prasugrel relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation*. 2009;119:2553–2544.
24. Mega JL, Wiviott SD, Sabatine MS. Cytochrome P-450 polymorphisms and response to clopidogrel: reply. *N Engl J Med*. 2009:360:2251.
25. Farid NA, Smith RL, Gillespie TA, Rash TJ, Blair PE, Kurihara A, Goldberg MJ. The disposition of prasugrel, a novel thienopyridine, in humans. *Drug Metab Dispos*. 2007;35:1096–1104.
26. Farid NA, Payne CD, Small DS, Winters KJ, Ernest CS II, Brandt JT, Darstein C, Jakubowski JA, Salazar DE. Cytochrome P450 3A inhibition by ketoconazole affects prasugrel and clopidogrel pharmacokinetics and pharmacodynamics differently. *Clin Pharmacol Ther*. 2007;81:735–741.
27. Small DS, Wrishko RE, Ernest CS II, Ni L, Winters KJ, Farid NA, Li YG, Brandt JT, Salazar DE, Borel AG, Kies KA, Payne CD. Prasugrel pharmacokinetics and pharmacodynamics in subjects with moderate renal impairment and end-stage renal disease. *J Clin Pharm Ther*. 2009;34: 585–594.
28. Small DS, Farid NA, Li YG, Ernest CS II, Winters KJ, Salazar DE, Payne CD. Pharmacokinetics and pharmacodynamics of prasugrel in subjects with moderate liver disease. *J Clin Pharm Ther*. 2009;34:575–583.
29. Small DS, Wrishko RE, Ernest CS II, Ni L, Winters KJ, Farid NA, Li YG, Salazar DE, Payne CD. Effect of age on the pharmacokinetics and pharmacodynamics of prasugrel during multiple dosing: an open-label, single-sequence, clinical trial. *Drugs Aging*. 2009;26:781–790.
30. Wrishko RE, Ernest CS, Small DS, Li YG, Weerakkody GJ, Riesmeyer JR, Macias WL, Rohatagi S, Salazar DE, Antman EM, Wiviott SD, Braunwald E, Ni L. Population pharmacokinetic analyses to evaluate the influence of intrinsic and extrinsic factors on exposure of prasugrel active metabolite in TRITON-TIMI 38. *J Clin Pharmacol*. 2009;49:984–998.
31. Sugidachi A, Ogawa T, Kurihara A, Hagihara K, Jakubowski JA, Hashimoto M, Nihira Y, Asai F. The greater in vivo antiplatelet effects of prasugrel as compared to clopidogrel reflect more efficient generation of its active metabolite with similar antiplatelet activity to that of clopidogrel's active metabolite. *J Thromb Haemost*. 2007;5:1545–1551.
32. Brandt JT, Payne CD, Wiviott SD, Weerakkody G, Farid NA, Small DS, Jakubowski JA, Naganuna H, Winters KJ. A comparison of prasugrel and clopidogrel loading doses on platelet function: magnitude of platelet inhibition is related to active metabolite formation. *Am Heart J*. 2007; 153:66.e9–66.e16.
33. Patti G, Colonna G, Pasceri V, Pepe LL, Montioaro A, Di Sciascio G. Randomized trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention: results from the ARMYDA-7 (Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty) study. *Circulation*. 2005;111: 2099–2106.
34. Lotrionte M, Biondi-Zoccai GG, Agostoni P, Abbate A, Angiolillo DJ, Valgimigli M, Moretti C, Meliga E, Cuisset T, Alessi MC, Montalescot G, Collet JP, Di Sciascio G, Waksman R, Testa L, Sangiorgi G, Laudito A, Trevi GP, Sheiban I. Meta-analysis appraising high clopidogrel loading in patients undergoing percutaneous coronary intervention. *Am J Cardiol*. 2007;100:1199–1206.
35. Dangas G, Mehran R, Guagliumi G, Caixeta A, Witzenbichler B, Aoki J, Peruga JZ, Brodie BR, Dudek D, Kornowski R, Rabbani LE, Parise H, Stone GW. Role of clopidogrel loading dose in patients with ST-segment elevation myocardial infarction undergoing primary angioplasty: results from the HORIZONS-AMI (Harmonizing Outcomes With Revascularizatiao and Steots io Acute Myocardial Infarction) trial. *J Am Coll Cardiol*. 2009;54:1438–1446.
36. Mathias S. A randomized comparison of a clopidogrel high loading and maintenance dose regimen versus standard dose and high versus low dose aspirin in 25,000 patients with acute coronary syndromes: results of the CURRENT OASIS 7 Trial. Presented at: European Society of Cardiology; August 29–September 2, 2009: Barcelona, Spain.
37. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, Angiolillo DJ, Hod H, Montalescot G, Miller DL, Jakubowski JA, Cairns R, Murphy SA, McCabe CH, Antman EM, Brauowald E. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation–Thrombolysis in Myocardial Infarction 44 trial. *Circulation*. 2007;116:2923–2932.
38. Wiviott SD, Antman EM, Winters KJ, Weerakkody G, Murphy SA, Behounek BD, Carney RJ, Lazzam C, McKay RG, McCabe CH, Braunwald E. Randomized comparison of prasugrel (CS-747, LY640315), a novel thienopyridine P2Y12 antagonist, with clopidogrel in percutaneous coronary intervention: results of the Joint Utilization of Medications to Block Platelets Optimally (JUMBO)-TIMI 26 trial. *Circulation*. 2005;111:3366–3373.
39. Jernberg T, Payne CD, Winters KJ, Darstein C, Brandt JT, Jakubowski JA, Naganuna H, Siegbahn A, Wallentin L. Prasugrel achieves greater inhibition of platelet aggregation and a lower rate of non-responders compared with clopidogrel in aspirin-treated patients with stable coronary artery disease. *Eur Heart J*. 2006;27:1166–1173.
40. Wiviott SD, Antman EM, Gibson CM, Montalescot G, Riesmeyer J, Weerakkody G, Winters KJ, Warmke JW, McCabe CH, Braunwald E. Evaluation of prasugrel compared with clopidogrel in patients with acute coronary syndromes: design and rationale for the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38). *Am Heart J*. 2006;152:627–635.
41. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2007;357:2001–2015.
42. Morrow DA, Wiviott SD, White HD, Nicolau JC, Bramucci E, Murphy SA, Bonaca MP, Ruff CT, Scirica BM, McCabe CH, Antman EM, Braunwald E. Effect of the novel thienopyridine prasugrel compared with clopidogrel on spontaneous and periprocedural myocardial infarction in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction 38: an applicatioo of the classification system from the universal definition of myocardial infarction. *Circulation*. 2009;119:2758–2764.
43. Wiviott SD, Braunwald E, McCabe CH, Horvath I, Kelasi M, Herrman JP, Van de Werf F, Downey WE, Scirica BM, Murphy SA, Antman EM. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. *Lancet*. 2008;371:1353–1363.
44. Wiviott SD, Braunwald E, Murphy SA, Antman EM. A perspective on the efficacy and safety of intensive antiplatelet therapy in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-thrombolysis in myocardial infarction 38. *Am J Cardiol*. 2008;101:1367–1370.
45. Unger EF. Weighing benefits and risks: the FDA's review of prasugrel. *N Engl J Med*. 2009;361:942–945.
46. Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FW, Goodman SG, Corbalan R, Purdy DA, Murphy SA, McCabe CH, Antman EM. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction 38. *Circulation*. 2008;118:1626–1636.

47. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, Antman EM. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. *Lancet*. 2009;373:723–731.
48. Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Antman EM. Prasugrel STEMI subgroup analysis reply. *Lancet*. 2009;373:1846–1848.
49. O'Donoghue M, Antman EM, Braunwald E, Murphy SA, Steg PG, Finkelstein A, Penny WF, Fridrich V, McCabe CH, Sabatine MS, Wiviott SD. The efficacy and safety of prasugrel with and without a glycoprotein IIb/IIIa inhibitor in patients with acute coronary syndromes undergoing percutaneous intervention: a TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction 38) analysis. *J Am Coll Cardiol*. 2009;54:678–685.
50. O'Donoghue ML, Braunwald E, Antman EM, Murphy SA, Bates ER, Rozenman Y, Michelson AD, Hautvast RW, Ver Lee PN, Close SL, Shen L, Mega JL, Sabatine MS, Wiviott SD. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. *Lancet*. 2009;374:989–997.
51. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med*. 2009;360:363–375.
52. Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dorrler K, Morath T, Schomig A, Kastrati A, von Beckerath N. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J*. 2009;30:916–922.
53. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Bensimon G, Funck-Brentano C, Montalescot G. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet*. 2009;373:309–317.
54. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009;302:849–857.
55. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, Freij A, Thorsen M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361:1045–1057.

KEY WORDS: anticoagulants ■ platelet aggregation inhibitors ■ platelets ■ thrombosis

Supplemental Tables

Table 1 (Supplemental): Clinical outcomes by weight at time of enrollment. Primary Endpoint = Cardiovascular death/myocardial infarction/stroke. Net Endpoint = All-cause death/myocardial infarction/stroke/TIMI major non-CABG bleed.

			Prasugrel	Clopidogrel		
Endpoint	Weight in Kilograms	N*	Event Rate	Event Rate	Hazard ratio	Interaction p-value
Primary EP (CVD/MI/stroke)	<60	668	10.5%	12.6%	0.86 (0.54-1.38)	0.55
	60- <70	1808	11.0%	13.7%	0.80 (0.61-1.04)	
	70- <80	3278	10.6%	12.6%	0.85 (0.69-1.04)	
	80- <90	3443	9.1%	10.9%	0.80 (0.65-1.00)	
	90- <100	2171	9.6%	11.1%	0.85 (0.65-1.11)	
	>=100 kg	2069	8.2%	11.2%	0.72 (0.54-0.96)	
TIMI major non-CABG bleed	<60	664	6.0%	3.5%	1.90 (0.90-4.02)	0.92
	60- <70	1791	2.1%	2.3%	0.96 (0.50-1.85)	
	70- <80	3255	2.2%	2.0%	1.24 (0.76-2.04)	
	80- <90	3420	2.6%	1.7%	1.41 (0.86-2.33)	
	90- <100	2154	1.5%	1.6%	0.96 (0.47-1.96)	
	>=100 kg	2052	2.3%	1.2%	1.75 (0.83-3.68)	
Net endpoint	<60	668	15.7%	15.9%	1.03 (0.69-1.53)	0.83
	60- <70	1808	13.2%	16.0%	0.81 (0.63-1.03)	
	70- <80	3278	12.5%	14.4%	0.87 (0.72-1.06)	
	80- <90	3443	11.3%	12.6%	0.86 (0.71-1.06)	
	90- <100	2171	11.8%	12.4%	0.93 (0.73-1.20)	
	>=100 kg	2069	10.8%	12.8%	0.82 (0.63-1.06)	

*Subjects for whom weight was not known at the time of enrollment are excluded from the analysis. N represents the intention to treat cohort for the primary and net endpoints and the safety cohort for TIMI major non-CABG bleeding

Table 2 (Supplemental): Clinical outcomes by age at time of enrollment. Primary Endpoint = Cardiovascular death/myocardial infarction/stroke. Net Endpoint = All-cause death/myocardial infarction/stroke/TIMI major non-CABG bleed.

			Prasugrel	Clopidogrel		
	Age in years	N*	Event Rate	Event Rate	Hazard ratio	Interaction p-value
Primary EP	<45	974	6.2%	11.0%	0.54 (0.34-0.86)	0.01
	45-49	1332	6.2%	9.3%	0.68 (0.45-1.01)	
	50-54	1902	6.7%	9.8%	0.69 (0.50-0.95)	
	55-59	2217	9.5%	11.6%	0.81 (0.62-1.05)	
	60-64	1897	9.9%	10.9%	0.88 (0.66-1.17)	
	65-69	1919	9.4%	11.0%	0.82 (0.62-1.09)	
	70-74	1558	12.2%	13.9%	0.91 (0.68-1.20)	
	>=75	1809	17.2%	18.3%	0.94 (0.75-1.18)	
TIMI major non-CABG bleed	<45	963	0.7%	1.0%	0.81 (0.18-3.61)	0.84
	45-49	1315	2.1%	0.8%	2.48 (0.88-6.96)	
	50-54	1890	1.8%	1.2%	1.47 (0.66-3.28)	
	55-59	2194	1.9%	1.8%	0.95 (0.49-1.85)	
	60-64	1883	2.8%	1.8%	1.57 (0.82-2.98)	
	65-69	1891	2.7%	2.3%	1.17 (0.64-2.12)	
	70-74	1536	2.6%	1.8%	1.35 (0.66-2.78)	
	>=75	1785	4.2%	3.4%	1.36 (0.81-2.27)	
Net endpoint	<45	974	6.7%	12.0%	0.54 (0.34-0.84)	0.02
	45-49	1332	8.9%	9.8%	0.89 (0.62-1.29)	
	50-54	1902	7.8%	10.8%	0.71 (0.53-0.97)	
	55-59	2217	11.5%	13.6%	0.83 (0.65-1.07)	
	60-64	1897	12.5%	12.3%	0.97 (0.75-1.26)	
	65-69	1919	12.0%	12.9%	0.90 (0.70-1.17)	
	70-74	1558	14.4%	16.5%	0.89 (0.68-1.15)	
	>=75	1809	21.5%	21.7%	0.99 (0.81-1.21)	

*N represents the intention to treat cohort for the primary and net endpoints and the safety cohort for TIMI major non-CABG bleeding

Exhibit F

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ACCF/AHA Clopidogrel Clinical Alert: Approaches to the FDA "Boxed Warning": A Report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the American Heart Association

Writing Committee Members, David R. Holmes, Jr, Gregory J. Dehmer, Sanjay Kaul, Dana Leifer, Patrick T. O'Gara and C. Michael Stein

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ACCF/AHA Clopidogrel Clinical Alert: Approaches to the FDA "Boxed Warning"

A Report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the American Heart Association

Endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons

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Preamble

The recent US Food and Drug Administration (FDA) "boxed warning" on clopidogrel raises important questions for practitioners and patients. The warning addresses the need for pharmacogenomic testing to identify patients' altered clopidogrel metabolism and thus their risk for a suboptimal clinical response to clopidogrel. Although there is an expanding database on genetic polymorphisms that may affect clopidogrel metabolism and thus clinical outcomes, there are no evidence-based data upon which todevelop specific recommendations on the role of genetic testing in routine care nor strategies proven to improve the safety/efficacy of specific pharmacologic approaches.

To provide guidance on this issue, the American College of Cardiology Foundation (ACCF) and the American Heart Asso-

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This document was approved by the American College of Cardiology Foundation (ACCF) Board of Trustees in June 2010, by the American Heart Association (AHA) Science Advisory and Coordinating Committee in June 2010, as well as endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons in June 2010.

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ciation (AHA) convened a writing committee. The ACCF and AHA adhere to a rigorous policy regarding relationships with industry and other entities (RWI) of authors and peer reviewers for clinical document development (see http://www.cardiosource.org/Science-And-Quality/Practice-Guidelines-and-Quality-Standards/Relationships-With-Industry-Policy.aspx). This policy requires that a majority of writing committee members have no *relevant* relationships with industry to this topic, a standard that has been achieved for this document as indicated in Appendix 1. In the spirit of full disclosure, *comprehensive* RWI (RWI not relevant to this document) for all authors is available online for public view. RWI restrictions are not applicable for participation in the external peer review process for clinical documents in order to ensure that a variety of constituencies/views inform the final document; however, all *relevant* reviewer RWI is published in Appendix 2 for the purpose of full transparency. In addition, reviewer affiliation for this document is recorded in Appendix 2, indicating participation of the following societies in the review process: the American Academy of Family Physicians, the American College of Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons. The ACCF and AHA believe this document will be helpful during a time when information on this topic is incomplete and continually changing. For some of the clinical issues in this document, final published data may not be available, in which case we have clearly identified this concern in the text. In addition to this document, an expert consensus document on the interaction of clopidogrel and proton pump inhibitors is in progress by the ACCF, American College of Gastroenterology, and AHA. Our organizations remain committed to providing guidance on key clinical issues to promote optimal patient care.

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1. Review of FDA Boxed Warning—What Did the FDA Say?

On March 12, 2010, the FDA approved a new label for clopidogrel with a "boxed warning" (Appendix 3) about the diminished effectiveness of the drug in patients with impaired ability to convert the drug into its active form.[1] This warning was the third FDA label change related to this issue in the last year. The boxed warning is based on the concern that the antiplatelet effect of clopidogrel depends primarily on its activation by the cytochrome P450 (CYP) system. Patients with decreased CYP2C19 function because of genetic polymorphisms metabolize clopidogrel poorly and have higher rates of cardiovascular events after acute coronary syndrome (ACS) and percutaneous coronary interventions (PCIs) than patients with normal CYP2C19 function. The warning also notes that tests are available to identify patients with genetic polymorphisms, and that alternative treatment strategies should be considered in poor metabolizers of the drug.

The new label emphasizes a single study of 40 healthy subjects (10 each with different degrees of CYP2C19 function—poor, intermediate, extensive, and ultrarapid) in a crossover design. Each group was randomized to a 300-mg loading dose (LD) followed by a 75-mg per day maintenance dose (MD), or a 600-mg LD followed by 150-mg per day MD, each for a total of 5 days (Appendix 3). After a washout period, subjects were crossed over to the alternate treatment. The chief findings were decreased active metabolite exposure and increased platelet aggregation in the poor metabolizers compared with the other groups. When poor metabolizers received the 600-mg LD followed by 150 mg daily MD, active metabolite exposure and antiplatelet response were greater than with the 300-mg LD and 75 mg per day MD regimen, but remained quantitatively less than the response in the extensive metabolizers when they received the 300 mg and 75 mg regimen. Two different assays for platelet function were used—platelet aggregation stimulated by 5 micromolar adenosine diphosphate (ADP) and the vasodilator-stimulated phosphoprotein phopsphorylation assay. Improvement in platelet inhibitory responses with higher-dose clopidogrel in poor metabolizers was apparent only with the former assay. There was no comment about statistical significance in the labeling material. Analysis of the final as yet unpublished data set of this study, which played a prominent role in the boxed warning, will be essential to a more complete understanding of the issues.

To fully understand the new label, it is necessary to consider the other background information upon which the label was developed. There are 3 major CYP2C19 genetic polymorphisms. CYP2C19*1 corresponds to normal function. CYP2C19*2 and CYP2C19*3 are loss-of-function alleles and explain most of the reduced function in those who are "poor metabolizers." CYP2C19*2 and *3 account for 85% and 99% of the nonfunctional alleles in whites and Asians, respectively (Appendix 3). Poor metabolizers have 2 loss-of-function alleles. Intermediate metabolizers have 1 copy of a loss-of-function allele and may also have decreased active metabolite levels and reduced antiplatelet effects when treated with clopidogrel, but the boxed warning only refers to poor metabolizers. The new label alludes to multiple retrospective, prospective randomized, and cohort clopidogrel studies that document increased major adverse cardiac event (MACE) rates in populations with genetic polymorphisms. Several cohort studies cited in prior FDA versions of the label and referred to in the most recent label have also shown variations in event rates that depend on CYP2C19 genotype.[2–4] As the new label notes, tests are now available to determine CYP2C19 genotypes for clinical purposes.

Conversely, it is also important to note what the label does not say. When the first of the label revisions was being developed in early 2009, the FDA proposed a recommendation for genotyping to identify patients with impaired CYP2C19 function and a comment stating that higher doses may be considered in these patients. After discussion with the manufacturer, however, no recommendation for genotyping was included in the label at that time. Instead, the initial 2009 revision (dated May 5, 2009) simply noted that "poor metabolizer status is associated with diminished response to clopidogrel" and that "the optimal dose for poor metabolizers

has yet to be determined".[5] The second revision in 2009 advised avoiding the use of clopidogrel "in patients with impaired CYP2C19 function due to known genetic polymorphisms or due to drugs that inhibit CYP2C19 activity" and added additional information about the interaction of clopidogrel and omeprazole.[6] The most recent revision, in March 2010, no longer specifically advises avoidance of clopidogrel in patients with known genetic polymorphisms of CYP2C19 but rather states that physicians should "consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers".[1] In addition, it should be noted that although it is assumed that it is the influence of the genotype on the phenotype of platelet reactivity that causes the increased rate of adverse clinical events, it remains possible that there may be other independent adverse effects of the genotype.

In the current warning, the moderate position of the FDA does not appear merely to be a reluctance to make strong recommendations about genetic testing, but rather to reflect an attempt to weigh the evidence and to give the prescriber more information. The FDA has made recommendations of different strengths related to genetic variations on multiple occasions[7] in recent years with some boxed warnings like those for carbamazepine and abacavir that explicitly recommend genetic testing and advise against generally treating patients with certain genotypes with these drugs.[8,9] In contrast, the FDA-approved label for warfarin mentions information about allelic variants that alter patient responsiveness to it, but does not include a "boxed warning" about this.[10]

1.1. Background and Significance of Boxed Warnings and How These Relate to the Clopidogrel Warning

It is important to understand when the FDA requires such a warning. The Code of Federal Regulations[10a] requires that "labeling shall be revised to include a warning as soon as there is reasonable evidence of an association of a serious hazard with a drug; a causal relationship need not have been proved. Special problems, particularly those that may lead to death or serious injury, may be required by the [FDA] to be placed in a prominently displayed box. The 'boxed warning' ordinarily shall be based on clinical data".[11]

The FDA leaves decisions about what to do with the information in boxed warnings up to individual clinicians.[11] It does not necessarily recommend a particular plan for how to deal with the information. It has been emphasized that the intent of information that the FDA puts into such a warning is to share the data with prescribers so they can be informed and make decisions based on patient-specific factors. The decision to perform CYP2C19 genetic testing is best made by the prescriber of the medication and the informed patient.

In brief, the clopidogrel boxed warning leaves the issue of whether to perform CYP2C19 testing up to the individual physician. It does not specifically require genetic testing or other changes in evaluation or treatment and does not imply that there are solid evidence-based reasons for such actions. Rather, it serves to make clinicians aware of the imperfect, but significant, knowledge that we have about genetic variations in response to clopidogrel and to emphasize that clinicians should use this knowledge to make decisions about how to treat individual patients.

2. Evidence on Variability to Clopidogrel Response

Clopidogrel, a thienopyridine $P2Y_{12}$ ADP receptor antagonist, requires bioactivation to its active metabolite (R130964) to inhibit platelet aggregation. There is substantial individual variability in response to clopidogrel, with inhibition of ADP-induced platelet aggregation ranging from less than 10% to almost complete inhibition of platelet aggregation with a wide distribution across this range, such that there is no dichotomous separation into "responders" and "nonresponders".[12] Nevertheless, a meta-analysis and other data suggest that residual platelet reactivity in patients receiving clopidogrel is associated with an increased risk of cardiac, cerebrovascular, and peripheral arterial events.[12–14] This variability may be due to pharmacokinetic (PK) or pharmacodynamic (PD) factors (ie, differences respectively in either kinetics/concentration of the active metabolite or in the response of platelets). The variability in clopidogrel PK and PD is due to several factors: demographic variables such as increased age and body mass index, comorbidities such as diabetes and dyslipidemia, and other factors that remain to be identified.[15] Although genetic variability also plays an important role in ADP-stimulated platelet aggregation in response to clopidogrel, known genetic and nongenetic factors explain only a portion of the majority of variation.[15]

2.1. Genetic Variability and Clopidogrel Response

2.1.1. CYP2C19 Variants

CYP2C19 plays an important role in the bioactivation of clopidogrel, a prodrug. Once absorbed, only approximately 15% of clopidogrel is bioactivated in the liver in a 2-step process that is mediated by several CYP450 isoenzymes (Figure 1).[16] Of these, CYP2C19 is responsible for approximately 45% of the first step (the formation of 2-oxo-clopidogrel) and approximately 20% of the final step—the generation of the pharmacologically active thiol metabolite. There are genetic polymorphisms in several CYP450 enzymes involved in the metabolism of clopidogrel, but variants in CYP2C19, particularly CYP2C19*2, are reproducibly associated with variability in clopidogrel active metabolite bioavailability, antiplatelet effects, and clinical outcomes.[2,15,17] The CYP2C19*2 variant encodes a nonfunctional protein. There are ethnic differences in its distribution; approximately 50% of Chinese, 34% of African Americans, 25% of Whites, and 19% of Mexican Americans carry at least 1 copy of the reduced function CYP2C19*2 allele.[18,19] Other genetic polymorphisms associated with impaired CYP2C19 activity and possibly adverse clinical events (CYP2C19*3, *4, *5, *8) are much less common in Whites, African Americans, and Hispanics.[18] In the randomized control TRITON–TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis In Myocardial Infarction 38), comparing clopidogrel with prasugrel in patients with ACS, CYP2C19*2 accounted for 95% of the subjects classified as carriers of a reduced CYP2C19 function allele.[17] The number of reduced function alleles is important:

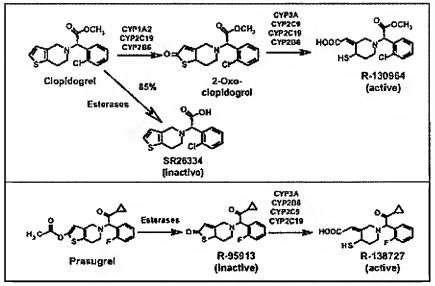

Figure 1. Schematic representation of the metabolism of clopidogrel and prasugrel. Reprinted with permission from Mega et al.[16]

individuals with 1 variant allele (intermediate metabolizers) had 26% to 31% lower exposure to the active metabolite of clopidogrel, and those with 2 genetic polymorphisms (poor metabolizers) had 46% to 55% lower exposure compared with those with no CYP2C19 polymorphisms.[17]

The effect of variant CYP2C19 alleles on clinical outcome in response to clopidogrel has been reported in multiple studies (Table 1).[20,21] All of these were cohort studies, with 1 being a genetic substudy derived from TRITON–TIMI 38. A significant association between the CYP2C19*2 polymorphism and an increased risk of major adverse cardiovascular events was reported in 5 of 7 studies. The risk ranged from a 53% relative increase in TRITON–TIMI 38[17] to an approximately 5-fold increase in a cohort study of young patients treated with clopidogrel after acute myocardial infarction (MI).[2] In the latter study, after multivariable analysis, CYP2C19*2 was the only factor independently associated with new cardiovascular events (hazard ratio [HR] 4.04 [95% confidence interval (CI) 1.81 to 9.02]; $P=0.0006$).[2] However, because none of the studies were randomized, the possibility of bias and confounding variables cannot be excluded. For example, patients who had an event were more likely to be receiving clopidogrel at baseline in some studies. Thus, a group of clopidogrel nonresponders may have been preselected and overrepresented in some studies. In addition, the scope of the genetic problem is not isolated to patients with 2 deficient alleles (homozygotes). This has important implications because of the higher prevalence of heterozygotes in the population. The data on positive and predictive risk in specific patient populations are incomplete.[22] Thus, caution must be observed in drawing definitive conclusions from these observational studies.

These clinical efficacy data mirror the effect of genetic polymorphisms on platelet function in both heterozygotes as well as homozygotes. Carriers of a CYP2C19*2 allele have been found to have an absolute reduction in platelet aggregation in response to clopidogrel that was 9 percentage points less than that of noncarriers.[17] Other studies[23] have noted that carriers of at least 1 reduced function CYP2C19 allele have less response to clopidogrel reflected as a higher residual platelet reactivity index. In a genome-wide association study performed in a homogenous population of healthy Amish—PAPI (Pharmacogenomics of Antiplatelet Intervention)—clopidogrel reduced ADP-induced platelet aggregation to 41%, 47%, and 65% of baseline in subjects with 0, 1, and 2 CYP2C19*2 alleles, respectively,[15] thereby exhibiting a gene-dose effect. However, even in this relatively homogenous population, CYP2C19*2 genotype accounted for only 12% of the variability in clopidogrel response.[15]

In contrast to clopidogrel, the FDA-approved drug, prasugrel, is oxidized to its active form in a single CYP-dependent step (Figure 1). In 238 healthy subjects tested, there was no significant decrease in the plasma concentrations of active metabolite or platelet inhibition in response to prasugrel in carriers versus noncarriers of at least 1 reduced function allele for the CYP genes tested (2C19, 2C9, 2B6, 3A5, 1A2).[16] Similar observations were reported in patients with stable coronary artery disease.[23] The association of these genetic variants with cardiovascular outcomes was examined in 1466 patients with ACS allocated prasugrel in TRITON-TIMI 38. No significant associations were found between any of the CYP genes tested and risk of cardiovascular death, MI, or stroke.[16] Ticagrelor, which is not yet approved, is a reversible, nonthienopyridine $P2Y_{12}$ receptor antagonist; it is not a prodrug, and does not require biotransformation.[24,25] The effect of genetic polymorphisms in CYP isoenzyme function or number for this drug remains incompletely defined. Other drugs, such as elinogrel (not yet approved), have also been studied.[26]

2.1.2. *Other Genetic Polymorphisms*

Other genetic variations may also affect the PK, PD, and clinical efficacy of clopidogrel.[27]

2.1.2.1. *ABCB1*

Intestinal absorption is limited by the P-glycoprotein efflux-transporter encoded by the adenosine triphosphate-binding

Table 1. CYP2C19*2 Polymorphisms and Cardiovascular Outcomes

Source, Year (Region)	Patients, n (Age, Years)	Disease	Clopidogrel Dosage	Duration of Follow-Up (Months)	Outcome (n)	Frequency of Genotype, n (%) *1/*1	*1/*2	*2/*2	RR (95% CI)	Adjustment
Trenk et al, 2008 *(Germany)*[21]	797 *(mean: 66.4)*	CAD	LD 600 mg MD 75 mg day^{-1}	12	Death and MI (24)	552 (69.3)	228 (28.6)	17 (2.1)	0.67 (0.25–1.78)†	None
Simon et al, 2009 *(France)*[22]	2178 *(mean: 70.1)*	AMI	LD 300 mg MD 75 mg day^{-1}	12	Death from any cause (225)	1561 (71.7)	564 (25.9)	53 (2.4)	0.89 (0.68–1.18)†	None
Collet et al, 2009 *(France)*[8]	259 *(18–45)*	MI	LD n.d. MD 75 mg day^{-1}	100	Death, MI, urgent coronary revascularization (26) Stent thrombosis (12)	186 (71.8)		73 (28.2)	5.38 (2.32–12.47) 6.04 (1.75–20.80)	BMI smoking, diabetes, stent implantation, STEMI, use of proton-pump inhibitors
Mega et al, 2009 *(United States)*[17]	1459 *(mean: 60.1)*	ACS	LD 300 mg MD 75 mg day^{-1}	15	Death from CV causes, MI, stroke (129) Stent thrombosis (18)	1064 (72.9)		395 (27.1)	1.53 (1.07–2.19) 3.09 (1.19–8.00)	None
Sibbing et al, 2009 *(Germany)*[9]	2485 *(mean: 66.5)*	CAD	LD 600 mg MD 75 mg day^{-1}	1	Stent thrombosis (17)	1805 (73)	633 (25)	47 (2)	3.81 (1.45–10.02)	Age, diabetes, ACS, type of stent
Giusti et al, 2009 *(Italy)*[9]	772 *(mean: 68.3)*	ACS	LD 600 mg MD 75 mg day^{-1}	6	Stent thrombosis + cardiac mortality (29) Stent thrombosis (24)	525 (68)	221 (28.6)	26 (3.4)	2.70 (1.00–8.42) 3.43 (1.01–12.78)	Residual platelet reactivity, traditional CV risk factors, clinical and procedural risk factors
Shuldiner et al, 2009 *(United States)*[15]	93‡ *(mean: 65)*	CAD	LD 300/600 mg day^{-1} MD 75 mg day^{-1}	12	MI, unplanned target and nontarget lesion revascularization, hospitalization, death from CV causes (a not reported)	66 (70.9)	27 (29.1)		3.40 (1.36–8.46)	Age, gender, race

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†Calculated from data taken from the original text.

‡Only patients who were still taking clopidogrel after 1 year.

ACS indicates acute coronary syndromes; AMI, acute myocardial infarction; BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; CV, cardiovascular; LD, loading dose; MD, maintenance dose; MI, myocardial infarction; n.d., no date; RR, risk ratio; and STEMI, ST-segment–elevation myocardial infarction.

Table 2. Pharmacodynamic Studies of Platelet Responsiveness to Different Clopidogrel Dosing Protocols

Study	Regimen	Metric	Results
ISAR-CHOICE[33] 60 elective PCI patients; C-naïve	C, 300, 600, 900 mg LD	Platelet aggregometry, active thiol metabolite of C	600 mg dose had highest active drug metabolite level and platelet suppression compared with the 300 mg dose.
von Beckerath et al.[34] 60 patients after successful PCI; 600 mg C load	C, 150 mg daily vs. 75 mg daily (MD)	30-d platelet function	C, 150 mg daily had more intense platelet inhibition.
OPTIMUS study[35] 40 patients with type 2 DM and documented suboptimal response to C	C, 150 mg daily vs. 75 mg daily (MD)	Repeat platelet function testing after 30 d	150 mg dose improved rates of platelet inhibition, but 60% of patients still had suboptimal C effect.
Fontana et al.[36] 81 patients with recent PCI and documented suboptimal platelet inhibition on C 75 mg daily	C increased to 150 mg daily C (MD)	Repeat platelet function testing after 15 d	C, 150 mg daily improved platelet inhibition.
PRINC trial[37] 60 patients undergoing PCI; C, 600 mg LD	2 h after initial C-load, either 600 mg C or placebo, then second randomization to 150 mg C vs. 75 mg C daily	Platelet inhibition at 2, 4, and 7 h, and 1 wk	600 mg load × 2 at 2 h apart produced better inhibition than 600 mg acutely; 150 mg daily results in better inhibition than 75 mg after 1 wk.
VASP-02[38] 153 patients undergoing elective PCI	C, 150 mg versus 75 mg daily for 4 wk; after 2 wk, platelet inhibition checked and low responders increased to 150 mg daily	Platelet inhibition at 2 and 4 wk	At 2 wk, 150 mg C produced better platelet inhibition. In low responders, 150 mg C improved platelet inhibition.
Price et al.[40] 45 volunteers	C, 300, 600, 900 mg LD	Platelet inhibition at baseline and 1 through 7 h	600 mg and 900 mg had more intense platelet inhibition than 300 mg, no difference between 600 mg and 900 mg.
Montalescot et al. (ALBION)[41] 103 patients with NSTEMI	C, 300, 600, 900 mg LD	ADP-induced IPA at 24 h	LDs greater than 300 mg provided greater antiplatelet effect than 300 mg

ADP indicates adenosine diphosphate; ALBION, Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis; C, clopidogrel; DM, diabetes mellitus; IPA, inhibition of platelet aggregation; ISAR-CHOICE, Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 Higher Oral Doses for Immediate Clopidogrel Effect; LD, loading dose; MD, maintenance dose; NSTEMI, non–ST-segment elevation myocardial infarction; OPTIMUS, Optimizing Antiplatelet Therapy in Diabetes Mellitus; PCI, percutaneous coronary intervention; PRINC, Plavix Response in Coronary Intervention; and VASP-02, Vasodilator-Stimulated Phosphoprotein-02 Randomized Study.

cassette containing gene ABCB1, also known as the multidrug resistant (MDR1) gene. Compared with noncarriers (wild-type or CC genotype), the bioavailability of clopidogrel is significantly reduced among patients receiving a 300- or 600-mg LD before elective PCI who have either 1 (CT genotype) or 2 (TT genotype) copies of the ABCB1 C3435T single nucleotide polymorphism.[28] In acute MI patients, the frequency of the variant TT genotype (TT 26%, CC 26%, CT 48%) was significantly higher among the 294 patients with an outcome event (death, nonfatal MI, or stroke at 1 year) compared with the 1914 patients without an event (29% versus 26%, $P=0.04$). In addition, patients with the TT genotype had significantly higher event rates at 1 year than those with the ABCB1 wild-type (CC) genotype (15.5% versus 10.7%; adjusted HR 1.72; 95% CI 1.20 to 2.47).[22] Patients who possessed 2 CYP2C19 loss-of-function alleles and at least 1 ABCB1 variant allele were at the highest risk for a primary outcome event (HR 5.31; 95% CI 2.13 to 13.20) compared with patients who had both CYP2C19 and ABCB1.[22] In another study of 2934 ACS patients, TT homozygotes had a 72% increased risk of the composite primary end point (cardiovascular death, MI, or stroke at 15 months) compared with either CC or CT patients (HR 1.72, $P=0.002$).[29] Additional information on the frequency and consequences of combined, functionally important genetic polymorphisms is required.

2.1.2.2. *Other CYP Isoenzymes*

The CYP3A4 and CYP3A5 enzymes also play a role in the conversion of clopidogrel to its active metabolite. In a substudy of healthy volunteers analyzed along with TRITON–TIMI 38, carrier status for a reduced function allele of CYP2C9, 3A5, and 1A2 was not associated with a consistent reduction of the PK or PD responses to clopidogrel. Carriers of a reduced function CYP2B6 allele, however, tended to have a lower plasma exposure to the active metabolite of clopidogrel and tended to have less reduction of platelet aggregation in response to clopidogrel.[16,17] One other study reported that subjects with the CYP3A5*3 allele had significantly decreased response to clopidogrel when it was combined with itraconazole, a CYP3A inhibitor, compared with CYP3A5*1 homozygotes.[30]

2.1.2.3. *P2Y$_{12}$ Receptor*

Studies have also assessed genetic variation in the gene encoding the P2Y$_{12}$ receptor (the binding site for clopidogrel metabolite). In

Table 3. Effect of Different Clopidogrel Dosing Protocols on Patient Outcomes

Study	Regimen	Metric	Results
ARMYDA-4 RELOAD[42a] 503 stable AP or non-STEMI ACS patients on chronic C for more than 10 d	600 mg load vs. placebo	30-d MACE defined as cardiac death, MI, or TVR	No benefit in overall cohort. In non-STEMI ACS patients, 600 mg load reduced MACE (16.3% to 6.4%); no change in MACE in stable AP.
HORIZONS-AMI[43] 3602 STEMI patients	600 mg vs. 300 mg C load	30-d MACE defined as all-cause death, stroke, reinfarction, unplanned revascularization for ischemia, or major bleeding	600-mg dose was an independent predictor of lower 30-d MACE
CURRENT OASIS-7* 25 098 ACS patients[41a]	High-dose C = 600-mg loading dose, then 150 mg for 7 d, then 75 mg daily to 30 d; standard dose C = 300 mg loading dose, then 75 mg daily to 30 d	30-d MACE defined as cardiovascular death, MI, or stroke	No benefit in overall cohort. In subgroup of 17,232 PCI patients, 15% reduction in MACE in high-dose group with a 42% reduction in definite ST, but increased bleeding

*Because the overall study was negative, the results obtained in the ACS subgroup in ARMYDA-4 RELOAD and PCI subgroup in CURRENT OASIS-7 should be considered hypothesis-generating only.

ACS indicates acute coronary syndromes; AP, angina pectoris; ARMYDA-4 RELOAD, Antiplatelet Therapy for Reduction of MYocardial Damage During Angioplasty-4 RELOAD trial; C, clopidogrel; CURRENT OASIS-7, Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Interventions trial; HORIZONS-AMI, Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction trial; MACE, major adverse cardiac events; MI, myocardial infarction; PCI, percutaneous coronary intervention; ST, stent thrombosis; STEMI, ST-segment elevation myocardial infarction; and TVR, target-vessel revascularization.

the FAST-MI (Registry on Acute ST–Elevation Myocardial Infarction) study, no association was found with clopidogrel responsiveness and the genetic polymorphism encoding the $P2Y_{12}$ receptor.[22] Other studies have also yielded similar results.[33]

3. Current Status of CYP2C19 Genotyping Assays

Given the increasing importance of genetic variations, there has been increasing interest in genetic testing to identify optimal strategies of care. This feature is a central component of the new clopidogrel boxed warning. Commercial assays are available from both research and clinical laboratories. Cross validation of the techniques used and their reliability, specificity, and reproducibility are extremely limited. While results of commercial assays can be applied, they are not available in the acute phases of patient care. Point-of-care assays for the common CYP2C19 polymorphisms are not available at this time. In addition, genetic polymorphisms with gain-of-function (CYP2C19*17), and uncommon alleles with reduced function (eg, CYP2C19*3, *4, *5) may affect clinical outcomes. An important patient care issue relates to the cost for these tests (approximately $500), which are typically not reimbursed by major payers. Alternatives to genetic testing focus on the phenotype—specifically, platelet function. Platelet function assays can measure the effect of ADP or $P2Y_{12}$ activation on platelet aggregation, receptor expression, or the level of intracellular molecules (eg, vasodilator-stimulated phosphoprotein phopsphorylation), thereby directly or indirectly measuring the platelet inhibitory effect of clopidogrel (ie, clopidogrel responsiveness or on-treatment reactivity). Additional clinical studies are underway to test whether altering therapy in response to residual high platelet reactivity after clopidogrel administration is associated with improved clinical outcomes.

4. Alternative Dosing Regimens for Clopidogrel

Evaluation of the different strategies developed and tested to overcome clopidogrel nonresponsiveness must consider the type of study, patient population, metrics of evaluation, and duration of follow-up. Each of these variables may affect interpretation of these data and the application of a specific therapeutic approach to an individual patient. There are few data on the inhibitory effect of alternative dosing regimens in CYP2C19 intermediate and/or poor metabolizers.

Several studies have evaluated the effect of different combinations of clopidogrel LDs and MDs on platelet aggregation, metabolites of clopidogrel, and other measures of platelet function[32–41] (Table 2). Some studies were performed specifically in patients with a documented suboptimal response to the usual dosing protocols for clopidogrel.[35,36,38] However, there are less data on the effect of alternative dosing regimens in intermediate and/or poor metabolizers and a lack of data supporting a change in therapy based on genotyping alone. In general, a 600-mg LD or double LD (second 600-mg dose 2 hours later) improves the degree of acute platelet inhibition.[33,37] Moreover, an MD of 150 mg daily results in a greater degree of platelet inhibition in many studies in patients with a reduced response to the usual 75-mg MD.[36–38] However, even at the higher dose, some patients do not reach an optimal level of platelet inhibition ex vivo.[35]

Fewer studies have examined patient outcomes after different clopidogrel dosing protocols including an additional 600-mg LD at the time of PCI in patients already receiving 75 mg daily,[40] 600-mg versus 300-mg LD in patients with ST-segment elevation MI undergoing primary PCI,[41] and 600-mg LD followed by 150 mg daily for 1 week in patients with ACS[41a] (Table 3). There was no overall benefit of reloading clopidogrel prior to PCI in patients already receiving chronic clopidogrel in the ARMYDA-4 RELOAD (Antiplatelet Ther-

apy for Reduction of MYocardial Damage During Angioplasty-4 RELOAD) study, although patients with ACS did appear to do better with the extra LD.[42] Because the overall study was negative, the results obtained in the ACS cohort should be considered hypothesis-generating only. In the HORIZONS-AMI (Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction) trial, a 600-mg dose was an independent predictor of lower 30-day MACE.[43] In the CURRENT–OASIS-7 (Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events–Optimal Antiplatelet Strategy for Interventions-7) trial, there was no increase in efficacy of double-dose versus standard-dose clopidogrel in the overall cohort.[41a] However, in patients undergoing PCI (nearly 75% of the overall cohort), MACE was significantly reduced but bleeding was also increased with double-dose clopidogrel.[41a] Because the overall study was negative, the results obtained in this postrandomization subgroup of patients undergoing PCI should be considered hypothesis-generating only.

Other strategies have been tested to overcome deficits in clopidogrel responsiveness. One approach is to add a third drug to aspirin and clopidogrel to further enhance platelet inhibition. Cilostazol acts via a different pathway to selectively inhibit phosphodiesterase type 3 and affects adenosine reuptake and nitric oxide PGI_2 production by endothelial cells.[44] It is approved for use in patients with peripheral vascular disease and claudication; thus, its use to enhance platelet inhibition in PCI patients is off-label. After stent placement, patients with persistently high platelet reactivity after a 300-mg LD of clopidogrel were randomized to receive either high-dose clopidogrel (150 mg daily) or cilostazol (100 mg twice daily) with the standard clopidogrel MD.[45] Adjunctive cilostazol intensified platelet inhibition to a greater degree than high-dose clopidogrel in these patients and also in a separate study of patients undergoing primary PCI for ST-segment elevation MI.[46] However, data on the effect of adjunctive cilostazol on clinical outcomes are conflicting. In a study of Asian patients with ACS, adjunctive cilostazol improved clinical outcomes at 6 months, but no platelet function testing was performed.[47] However, in the recently reported CILON-T (Efficacy of Cilostazol on Ischemic Complications After DES Implantation) study, adjunctive cilostazol did not result in a reduction in cardiovascular events at 6 months despite improved platelet inhibition.[47a] In addition, drug interactions and gastrointestinal intolerance with cilostazol may be problematic. Other isolated studies show enhanced platelet inhibition or a reduction in cardiovascular events with the addition of omega-3 fatty acids[48] or specific glycoprotein IIb/IIIa inhibitors (abciximab and tirofiban) administered acutely with aspirin and clopidogrel.[49,50] However, none of these studies provide substantial proof of efficacy in large populations at risk.

The other approach has been to substitute a newer, more potent platelet inhibitor drug for clopidogrel. Prasugrel, recently approved for clinical use, still requires single-step hepatic conversion to an active metabolite before binding to the platelet $P2Y_{12}$ receptor. It thus far appears to have very few poor responders in patients with stable coronary artery disease[51] and in patients with ACS.[52] Standard dosing of prasugrel (60 mg loading, 10 mg daily) is associated with more potent platelet inhibition than clopidogrel even at high doses (600 mg loading, 150 mg daily).[51,53–55] Moreover, when administered chronically, a 10-mg daily dose of prasugrel provides better inhibition of platelet function than 75 mg or 150 mg of clopidogrel daily. Enhanced platelet inhibition with prasugrel was documented in a small substudy of TRITON–TIMI 38[52]; significantly reduced rates of ischemic events compared with those seen with clopidogrel, including stent thrombosis, were reported in TRITON–TIMI 38.[56] There was, however, an increased rate of major bleeding, including life-threatening bleeding. The 3 groups at highest risk for bleeding in TRITON–TIMI 38 included those greater than 75 years of age, with body weight less than 60 kg, and with a history of stroke or transient ischemic attack. Prasugrel ***should not*** be used in patients with qualifying ischemic stroke. In an attempt to balance the excess risk of bleeding associated with prasugrel with its benefit in reducing stent thrombosis (particularly early after stent placement), some clinicians have used an empiric strategy of prasugrel for 1 month then followed by a switch to a standard-dose clopidogrel.

Ticagrelor, although not yet available for clinical use, is an oral, reversible $P2Y_{12}$ receptor antagonist that blocks ADP-induced platelet aggregation and does not require metabolic activation.[24] Compared with clopidogrel in a large trial of patients with ACS, ticagrelor significantly reduced the rate of the primary composite end point of death from vascular causes, MI, or stroke.[25] Ticagrelor reduced the individual components of death from vascular causes and MI, but not the rate of stroke. While there was no increase in the rate of overall major bleeding, there was an increase in the rate of nonprocedure-related bleeding. Specifically in patients with a planned invasive strategy, ticagrelor had significant and clinically relevant reductions in cardiovascular and total deaths, MI, and stent thrombosis, without an increase in risk of major bleeding.[57] This drug has also been found to be effective in improving platelet inhibition in patients who are nonresponders to clopidogrel.[58]

5. Review of Ongoing Trials

Testing for genetic polymorphisms received considerable emphasis in the boxed warning. While CYP2C19 genetic polymorphisms have been shown in several studies to reduce clopidogrel metabolism and its PD effect and clinical effectiveness, there are no prospective studies demonstrating a clinical benefit to personalizing antiplatelet therapy based on genotype analysis. The study upon which the FDA issued the boxed warning and based its statement "to consider alternative treatment strategies" is a small unpublished crossover trial that evaluated PK and antiplatelet responses to clopidogrel in 40 healthy subjects. How these data should translate into clinical practice remains the focus of ongoing studies. Several studies of different populations, sizes, degree of methodological rigor, and follow-up are currently underway or being planned to evaluate the role of pharmacogenetic (CYP2C19) profiling of patients in their PK (active metabolite exposure) and PD (platelet function assays) responses to clopidogrel (Table 4). Two of these ongoing studies are exploring the influence of CYP2C19 in the drug interaction

Table 4. Ongoing Trials Evaluating Antiplatelet Therapy Tailored by Genotyping and/or Phenotype Assessment

Study	Design	No. of Patients	Population	Selection Criterion	Outcome	Follow-Up
Trials Evaluating Pharmacodynamic and/or Pharmacokinetic Outcomes						
GIFT (NCT00992420) PI: M.J. Price	Observational, prospective cohort study (GRAVITAS substudy) (PD study)	Up to 2000	Stable CAD or NSTEMI ACS undergoing DES	Patients with high residual platelet activity (HRPA) 12- to 24-h post-DES randomized to: 1) standard 75 mg clopidogrel, or 2) high-dose clopidogrel (additional 600 mg followed by 150 mg daily)	Association of CYP2C19 genotype with RPA (VerifyNow) on standard dose of clopidogrel or incremental change RPA on high-dose clopidogrel	6 mo
Clopidogrel Pharmacogenomics Project (NCT01097343) PI: J. Dharmavaram PI: J.S. Rossi	Randomized, open-label, crossover, phase 0 (PD/PK study)	200	Stable CAD	Screen for CYP2C19*2 LOF allele; randomize eligible patients to clopidogrel 75 mg or 150 mg daily × 30 d and then crossover	Change in RPA (VerifyNow, optical aggregometry); measurement of active metabolites	90 d
CLOVIS-2 (NCT00822666) PI: J.-P. Collet PI: G. Montalescot	Randomized, open-label, phase III, crossover (PD/PK study)	120	Post-MI, <45 y and enrolled in AFIJI registry	Comparison of 2 loading strategies of clopidogrel (300 mg vs. 900 mg) in 2 genetic profiles: wild-type 2C19*1 and carriers of 2C19*2 (homozygous or heterozygous)	Inhibition of RPA (IRPA) by optical aggregometry; measurement of active metabolites	6 h postclopidogrel loading dose
Role of CYP2C19 Polymorphism in the Drug Interaction Between Clopidogrel and Omeprazole (NCT01094275) PI: S. Nadipalli PI: T. Delao	Observational, case-crossover, phase IV (PD/PK study)	75	Healthy volunteers	Subjects with CYP2C19*2/*3 LOF allele, and age- and gender-matched wild-type control randomized to clopidogrel + omeprazole vs. clopidogrel × 1 wk, and crossover	Platelet inhibitory response to clopidogrel; measurement of active metabolites	3 wk
ELEVATE-TIMI 56 PI: J.L. Mega[58a]	Randomized treatment sequence (PD study)	275	Stable CAD	Patients on clopidogrel 75 mg and genotyped for CYP2C19 alleles will be treated with biweekly dose of clopidogrel "(75 mg to 300 mg daily, depending on genotype)."	Change in RPA (VerifyNow, VASP)	8 wk
PREDICT Pilot Study (NCT00747656) PI: M.J. Price	Observational prospective cohort (PD study)	42	Stable CAD on clopidogrel therapy	Patients with HRPA on clopidogrel 75 mg and genotyped for CYP2C19 alleles treated with double-dose clopidogrel (150 mg)	Change in RPA (VerifyNow)	7 d
ACCEL-2C19 (NCT01012193) PI: Y.-H. Jeong	Randomized, active-control, single-blind (PD study)	134	Stable CAD, elective PCI	Patients genotyped for CYP2C19 variants randomized to high-dose clopidogrel (150 mg) + ASA 200 mg vs. cilostazol 100 mg bid + 75 mg clopidogrel + ASA 200 mg (triple therapy)	Inhibition of maximum platelet aggregation (optical aggregometry; VerifyNow)	30 d
ACCELAM2C19 (NCT00915733) PI: I.-S. Kim	Randomized, active-control, open-label (PD study)	80	Acute MI, post-PCI	Patients genotyped for CYP2C19 variants randomized to high-dose clopidogrel (150 mg) + ASA 100 mg vs. cilostazol 100 mg bid + 75 mg clopidogrel + ASA 100 mg (triple therapy)	Inhibition of maximum platelet aggregation (optical aggregometry; VerifyNow)	30 d

(*Continued*)

Table 4. Continued

Study	Design	No. of Patients	Population	Selection Criterion	Outcome	Follow-Up
ACCEL-2C19 (NCT00891670) PI: Y.-H. Jeong	Randomized, active-control, open-label (PD study)	80	Stable CAD, Elective PCI	Patients genotyped for CYP2C19 variants randomized to high-dose clopidogrel (150 mg) + ASA 100 mg vs. cilostazol 100 mg bid + 75 mg clopidogrel + ASA 100 mg (triple therapy)	Maximum platelet aggregation (optical aggregometry; VerifyNow)	30 d
SPICE (NCT00930670) PI: U. Dery PI: G. Rossignol	Randomized, active-control, open-label (PD study)	320	Stable CAD, elective PCI with BMS	Subjects genotyped for CYP2C19 alleles and treated with clopidogrel randomized to statin + PPI or statin + H2RA	Change in RPA (optical aggregometry; VASP) Death, MI, stroke, or ischemia-driven TVR (secondary end point)	30 and 60 d
Influence of CYP2C19 Genetic Variants on Clopidogrel In Healthy Subjects (NCT00413608) PI: J.S. Hulot	Observational, active-control, open-label (PD/PK study)	30	Healthy volunteers	Patients genotyped for CYP2C19 variants with HRPA on clopidogrel 75 mg ("bad responders") will be given 150 mg clopidogrel and compared with results of 75 mg clopidogrel in "good responders"	Change in RPA (optical aggregometry); measurement of active metabolites	7 d
Trials Evaluating Clinical Outcomes						
GeCCO (NCT00995514) PI: E.J. Stanek	Observational, prospective cohort, open-label, active control, noninferiority study (outcome study)	14 600	Recent NSTEMI or STEMI ACS with or without primary or delayed PCI	Genotype-guided comparison of clopidogrel (75mg daily) in extensive metabolizers (CYP2C19*1/*1) and prasugrel (5 mg or 10 mg daily)	CV death, nonfatal MI, or nonfatal stroke	6 mo

ACCEL-2C19, Adjunctive Cilostazol Versus High Maintenance-dose Clopidogrel According to Cytochrome 2C19 Polymorphism; ACCELAMI2C19, Adjunctive Cilostazol Versus High Maintenance-dose ClopidogrEL In Acute Myocardial Infarction (AMI) Patients According to CYP2C19 Polymorphism; ACS, acute coronary syndromes; AFIJI, Appraisal of Risk Factors in Young Ischemic Patients Justifying Aggressive Intervention; ASA, acetylsalicylic acid; BMS, bare-metal stent; CAD, coronary artery disease; CLOVIS-2, Clopidogrel and Response Variability Investigation Study 2; CV, cardiovascular; CYP, cytochrome P450; DES, drug-eluting stent; GeCCO, Genotype Guided Comparison of Clopidogrel and Prasugrel Outcomes Study; GIFT, Genotype Information and Functional Testing Study; GRAVITAS, Gauging Responsiveness With A VerifyNow Assay-Impact On Thrombosis And Safety; HRPA, high-residual platelet activity; LOF, loss-of-function; MI, myocardial infarction; NSTEMI, non–ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; PD, pharmacodynamic; PI, primary investigator; PK, pharmacokinetic; PPI, proton pump inhibitor; PREDICT, Prehospital Evaluation and Economic Analysis of Different Coronary Syndrome Treatment Strategies; RPA, residual platelet activity; SPICE, Evaluation of the Influence of Statins and Proton Pump Inhibitors on Clopidogrel Antiplatelet Effects; STEMI, ST-segment elevation myocardial infarction; TVR, target-vessel revascularization; and VASP, vasodilator-stimulated phosphoprotein phosphorylation.

between clopidogrel and proton pump inhibitors, 1 with coadministration of statins (the SPICE [Evaluation of the Influence of Statins and Proton Pump Inhibitors on Clopidogrel Antiplatelet Effects] trial) and 1 without (the Influence of CYP2C19 Genetic Variants on Clopidogrel in Healthy Subjects study [NCT00413608]). Three trials are evaluating the role of triple antiplatelet therapy with cilastozol plus aspirin and standard dose clopidogrel versus aspirin plus high-dose clopidogrel in CYP2C19-genotyped patients with stable and ACS (ACCEL-2C19 [Adjunctive Cilostazol Versus High Maintenance-dose Clopidogrel According to Cytochrome 2C19 Polymorphism] [NCT01012193], ACCELAMI2C19 [Adjunctive Cilostazol Versus High Maintenance-dose Clopidogrel in Acute Myocardial Infarction (AMI) Patients According to CYP2C19 Polymorphism] [NCT00915733], and ACCEL2C19 [Comparison of Platelet Inhibition With Adjunctive Cilostazol Versus High Maintenance-Dose Clopidogrel According to Hepatic Cytochrome 2C19 Allele (CYP2C19) Polymorphism] [NCT00891670] trials). However, use of a surrogate end point (ex vivo platelet function assays) in these trials may limit the clinical significance of these data, as the causal relationship between laboratory measures of platelet function, and clinically relevant cardiovascular end points remains unproven. In addition, it is unknown whether platelet function tests are complementary to genotyping. Only 1 large observational, open-label study is examining genotype-guided comparison of clopidogrel 75 mg daily in extensive metabolizers (CYP2C19*1/*1) and prasugrel 5 mg or 10 mg daily (without complementary point-of-care testing of platelet function) on cardiovascular outcomes in patients with ACS (GeCCO [Genotype Guided Comparison of Clopidogrel and Prasugrel Outcomes Study] [NCT00995514]). Finally, a prospective randomized study—the PAPI-2 (Pharmacogenomics of Antiplatelet Intervention-2) trial—examining the role of CYP2C19*2 variant in influencing the PK, PD, and clinical response to clopidogrel is planned to be launched in the near future (A. Shuldiner, personal communication, April 2010).

Table 5. Trials Evaluating Antiplatelet Therapy Tailored by Pharmacodynamic Assessment

Study	Design	No. of Patients	Population	Selection Criterion	Outcome	Follow-Up
GRAVITAS (NCT00645918) PI: M.J. Price	Randomized, placebo-control, multicenter	2800	Stable CAD or NSTEMI ACS undergoing PCI (DES)	Patients with HRPA 12- to 24-h post-DES randomized to: 1) standard 75 mg clopidogrel; or 2) high-dose clopidogrel (additional 600 mg followed by 150 mg daily)	CV death, nonfatal MI, or definite/probable stent thrombosis	6 mo
ARCTIC (NCT00827411) PI: G. Montalescot PI: J.-P. Collet	Randomized, active-controlled, open label, phase IV, multicenter	2500	Stable CAD, elective PCI	Patients post-DES randomized to: 1) standard dose clopidogrel plus aspirin (conventional arm); or 2) adjusted-dose clopidogrel plus aspirin based on HRPA (monitoring arm)	Death, nonfatal MI, stroke, urgent TVR, or stent thrombosis	1 y
TRIGGER-PCI (NCT00910299) Sponsor: Eli Lilly	Randomized, active-control, double blind, phase II, multicenter	2150	Stable CAD, elective PCI	Patients 24 h post-DES and 2 to 7 h postclopidogrel and HRPA randomized to: 1) prasugrel 60 mg load/10 mg daily; or 2) clopidogrel 75 mg daily	CV death or nonfatal MI	6 mo
DANTE (NCT00774475) PI: G.F. Gensini PI: R. Marcucci	Randomized, active-control, open-label, phase III, multicenter	442	NSTEMI ACS undergoing PCI	Patients with HRPA randomized to: 1) clopidogrel 75 mg maintenance (standard dose); or 2) clopidogrel 150 mg maintenance (high dose)	CV death, nonfatal MI, or TVR	6 and 12 mo

ACS indicates acute coronary syndromes; ARCTIC, Double Randomization of a Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and a Interruption Versus Continuation of Double Antiplatelet Therapy; CAD, coronary artery disease; CV, cardiovascular; DANTE, Dual Antiplatelet Therapy Tailored on the Extent of Platelet Inhibition; DES, drug-eluting stent; GRAVITAS, Gauging Responsiveness With A VerifyNow Assay-Impact On Thrombosis And Safety; HRPA, high residual platelet activity; MI, myocardial infarction; NSTEMI, non–ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; PI, primary investigator; TRIGGER-PCI, Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel; and TVR, target-vessel revascularization.

Whether the promise of pharmacogenetic testing in tailoring antiplatelet therapy to the individual patient will be fulfilled awaits the completion of these studies.

Platelet function testing used to tailor antiplatelet therapy has also received considerable interest. Although this field suffers from a surfeit of specific assays, definitions, and protocols, it has the advantage that point-of-care testing is currently available. There are currently 4 ongoing trials testing the hypothesis that tailoring antiplatelet therapy based on platelet responsiveness assessed in an ex vivo $P2Y_{12}$ assay will improve cardiovascular outcomes. The details of these trials are summarized in Table 5. Patients with high residual platelet activity are randomly allocated to standard-dose versus high-dose clopidogrel in 2 trials (GRAVITAS [Gauging Responsiveness With A VerifyNow Assay-Impact On Thrombosis And Safety] and DANTE [Dual Antiplatelet Therapy Tailored on the Extent of Platelet Inhibition]), and to standard-dose clopidogrel versus prasugrel in 1 trial (TRIGGER-PCI [Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel]), while the ARCTIC Double Randomization of a (Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy) trial is evaluating dose adjustment of dual antiplatelet therapy with aspirin and clopidogrel on the basis of biological monitoring compared with the conventional, unmonitored strategy. The primary outcome in these trials is the time to first occurrence of cardiovascular complications including cardiovascular death, nonfatal MI, nonfatal stroke, stent thrombosis, or target vessel revascularization at 6 or 12 months. The routine clinical use of platelet function testing to screen clopidogrel-treated patients undergoing PCI in order to maximize efficacy while maintaining safety may be supported only after these clinical trials are completed.

Pharmacogenetic and/or PD profiling could potentially offer a tool to identify a priori patients in whom an alternative antiplatelet approach to standard-dose clopidogrel would decrease ischemic events. Options include acute administration of glycoprotein IIb/IIIa inhibitors or longer-term use of higher-dose clopidogrel, cilastozol, prasugrel, ticagrelor, or new agents such as elinogrel.[26] Although the effectiveness of ticagrelor in improving cardiovascular outcomes was demonstrated in the PLATO (Platelet Inhibition And Patient Outcomes) trial, this drug is not approved by the FDA. Another promising drug, elinogrel, has only been evaluated in a phase 2 study. At the present time, prasugrel is the only new agent approved to support PCI in patients with ACS. It must be kept in mind, however, that the use of prasugrel in high-risk genotype patients after elective PCI has not been studied. With regard to clopidogrel dose adjustment in patients with high platelet reactivity on standard clopidogrel treatment, the results are mixed. Two studies yielded improved outcomes of major adverse cardiovascular events or stent thrombosis with this approach.[59,60] In contrast, 1 case

series of patients with prior MI who subsequently developed stent thrombosis questioned the strategy of increasing clopidogrel dose in carriers of CYP2C19*2 as time-consuming and largely ineffective at providing adequate platelet inhibition (although prasugrel was able to suppress platelet aggregation successfully).[61] Even if the relationship between genetic polymorphisms and ischemic risk is well established, the effect on bleeding remains to be elucidated. It is possible that the relationship between genetic polymorphisms and antiplatelet effect is somewhat different for thrombosis and bleeding. Higher levels of active-metabolite generation may not necessarily translate into an optimal benefit-risk balance. As documented in the preceding text, it is difficult to interpret some current studies[41a] because of lack of concordance between the main trial findings and those obtained in patient subsets. A fundamental issue remains that it is not known whether treatment decisions predicated on the results of either genotyping or phenotyping information can impact optimization of either clinical efficacy and/or safety. Genotyping of patients enrolled in the studies listed in Table 4, and the ONSET/OFFSET (Randomized Double-Blind Assessment of the ONSET and OFFSET of the Antiplatelet Effects of Ticagrelor Versus Clopidogrel in Patients With Stable Coronary Artery Disease) study,[62] which demonstrated superior antiplatelet effects of ticagrelor compared with higher LD of clopidogrel, or the RESPOND (Response to Ticagrelor in Clopidogrel Nonresponders and Responders and Effect of Switching Therapies) study,[58] which showed similar platelet inhibitory effects of ticagrelor in clopidogrel responders and nonresponders alike, will likely yield valuable insights in this regard.

In summary, larger and longer-term prospective studies that include cardiovascular event outcomes are necessary to optimize predictive algorithms that may include genetic and/or platelet function testing, and their use to individualize $P2Y_{12}$ inhibitor therapy. However, it is important to recall that CYP2C19 polymorphism accounts for only approximately 12% of variability in clopidogrel platelet response[15] and that the positive predictive value of CYP2C19 loss-of-function genetic polymorphisms for clinical events is estimated to be between 12%[16] and 20%[2] in patients with ACS undergoing PCI. Even if slightly better than the positive predictive values observed with the point-of-care $P2Y_{12}$ assay in a similar population (12%),[63–65] the predictive accuracy of these genetic polymorphisms is still low. Thus, improvement in prediction of future cardiovascular events in patients receiving antiplatelet therapy will likely benefit from development of a global risk assessment score based on traditional demographic, clinical, and procedural risk factors, genetics, and biological information rather than any single test result.

6. Conclusions

6.1. Issues for Consideration

The information on the pharmacogenomics that has formed the basis for the recent boxed warning on clopidogrel is of great importance in understanding the issues related to variability in clinical outcomes of patients with both acute and chronic coronary artery disease; in addition, this information may have applicability for patients with stroke and peripheral arterial disease, although there are no robust data in these populations. There are several critical issues that require careful consideration. As noted in the preceding text, CYP2C19 polymorphism accounts for only approximately 12% of variability in clopidogrel platelet response, and the positive predictive value of CYP2C19 loss-of-function genetic polymorphisms is estimated to be between 12% and 20% in patients with ACS undergoing PCI. In addition, there is no prospective randomized evidence to support genotyping, a direct effect of genetic polymorphisms cannot be excluded, and there is a larger body of evidence to support platelet function testing as a risk stratifier for adverse events. These issues must be considered in the context that there are multiple unknown factors including, most importantly, the fact that the specific role of an individual genetic polymorphism in influencing outcome for the individual patient remains unknown.

1. Guideline adherence remains the cornerstone of care. Clinical judgment is required to assess individual risk and variability in response to clopidogrel. While imperfect, such judgment is essential. In addition to consideration of evidence-based guidelines, it is crucial to emphasize patient compliance with the prescribed antiplatelet regimen. Given the large interindividual variability in response to clopidogrel resulting from both clinical and genetic factors, the issues of genotyping and measurement of platelet inhibition have been raised, particularly in patients felt to be at highest risk for adverse events and in patients who have already had an adverse event despite compliance with regimens of aspirin and clopidogrel (coronary artery disease patients) or clopidogrel monotherapy (cerebrovascular ischemia patients).
2. Information on patients at risk for poor outcomes with ACCF/AHA and AHA Stroke Council Guideline-recommended therapy continues to accumulate. Some patients are identified because they have experienced an adverse outcome, such as stent thrombosis, while other patients may be considered to be at increased risk of a subsequent adverse outcome, including stent thrombosis, MI, ischemic stroke, and vascular death. This latter consideration may be based on clinical characteristics such as diabetes mellitus, chronic renal failure, or angiographic variables (eg, diffuse 3-vessel or left-main coronary artery disease or multifocal cervicocerebral atherosclerotic disease). In the future, profiling high-risk populations may include consideration of the frequency of the genetic penetration of genetic polymorphisms in that specific population.
3. Genetic variability in CYP enzymes may affect platelet function and has been associated with adverse patient outcomes in registry experiences and clinical trials. Although CYP2C19*2 is the most common genetic variant reproducibly associated with impaired responses to clopidogrel, the specific role of the individual genetic polymorphisms in impacting outcome remains to be determined (eg, the importance of CYP2C19*2 versus *3 or *4 for a specific patient).

In addition, there are other genetic polymorphisms such as ABCB1 that may also contribute to variation in the response of individual patients to clopidogrel.

Information about the predictive value of pharmacogenomic testing is very limited, but is the focus of multiple ongoing studies. The design of such studies in terms of specific tests and patient populations (eg, acute care versus chronic care settings) will have major implications for the role of testing. A related issue is whether the risk from a given individual's genomic profile changes over time, depending on the specific clinical scenario (eg, ACS versus stable angina pectoris, PCI versus medical therapy, small vessel versus large artery, atherosclerotic ischemic stroke, or carotid stenting versus medical therapy), is relevant. This question has yet to be resolved.

4. The answer to the specific question of the role of genotyping in everyday practice remains unknown at the present time. Although the boxed warning does not mandate testing, proponents would argue that there are common genetic polymorphisms that have been shown to affect the platelet response to clopidogrel as well as its clinical effectiveness in both randomized clinical trials and registry experiences. In addition, there are commercially available genetic tests that can determine CYP2C19 genotype variants although the turn-around time varies as does the cost, which is not routinely reimbursable at this time. Advocates argue that given the magnitude of the potential clinical consequences of suboptimal platelet inhibition based on genetic variation, assessment of genotypes would be justifiable. In contrast, opponents believe that there is no definitive proof at the current time that intervening on the basis of genotype improves outcome, and that there are other factors that may be more important. In addition, they would raise the question of whether genotyping should be confined to loss-of-function CYP2C19*2 or *3 (poor metabolizers), or be extended to other variants including the gain-of-function CYP2C19*17 variant (hyper-rapid or ultrarapid metabolizers). As part of this argument, opponents note that the predictive performance of CYP2C19 variant is low, ranging from 12% to 20%, and raise the question of what to do when variant genotype information is identified in patients with no clinical events. Finally, they would note that there are no point-of-care genotyping tests, which severely limits the usefulness of these data in the acute care setting. Currently, there are studies underway or in the planning stages that will address these issues to varying degrees. Despite the gaps in current knowledge, both clinicians and patients need to be aware of genetic polymorphisms that may modulate clopidogrel responsiveness and cause MACE. It is important to emphasize again that in the most recent labeling for clopidogrel, the FDA only informs physicians and patients that genetic testing is available; it neither mandates, requires, nor recommends genetic testing, thereby allowing for flexibility in clinical decisions.
5. Given the concerns about the mortality and morbidity that may be attributable to an inadequate response to antiplatelet therapy, there are a number of alternative approaches to standard guideline-based care with clopidogrel. New agents and new strategies have been used clinically and tested in a wide variety of situations. New agents such as prasugrel and ticagrelor, which are not affected by CYP2C19 genetic variants, have been found to be more effective than standard-dose clopidogrel. This relates to the PK characteristics of these newer agents. In very high-risk clinical circumstances (eg, prior stent thrombosis) such agents may be considered alternatives to standard ACCF/AHA and AHA Stroke Council Guideline therapy. This is particularly important in any patient suspected of treatment failure to standard-dose clopidogrel. Other treatment strategies are also being tested, including increased clopidogrel dosing or the addition of a third drug such as cilostazol to aspirin and clopidogrel. In the setting of stroke or transient ischemic neurologic symptoms, the combination of aspirin and extended release dipyridamole and aspirin monotherapy are alternatives recommended by the AHA Stroke Council guidelines for secondary prevention of stroke.[66]

6.2. Recommendations for Practice

Consideration of these critical issues leads to the following recommendations for clinicians:

1. Adherence to existing ACCF/AHA guidelines for the use of antiplatelet therapy should remain the foundation for therapy. Careful clinical judgment is required to assess the importance of the variability in response to clopidogrel for an individual patient and its associated risk to the patient. While imperfect, such careful judgment is essential.
2. Clinicians must be aware that genetic variability in CYP enzymes alter clopidogrel metabolism, which in turn can affect its inhibition of platelet function. Diminished responsiveness to clopidogrel has been associated with adverse patient outcomes in registry experiences and clinical trials.
3. The specific impact of the individual genetic polymorphisms on clinical outcome remains to be determined (eg, the importance of CYP2C19*2 versus *3 or *4 for a specific patient), and the frequency of genetic variability differs among ethnic groups. This has particular relevance related to the frequency of homozygotes, which occurs in approximately 2% of the population, versus heterozygotes, which occurs in approximately 30% of the population, both of whom may have increased risk.
4. Information regarding the predictive value of pharmacogenomic testing is very limited at this time; resolution of this issue is the focus of multiple ongoing studies. The selection of the specific test, as well as the issue of reimbursement, are both important additional considerations.
5. The evidence base is insufficient to recommend either routine genetic or platelet function testing at the present time. There is no information that routine testing improves outcome in large subgroups of patients. In addition, the clinical course of the majority of patients treated with clopidogrel without either genetic testing or functional testing is excellent. Clinical judgment is required to assess clinical risk and variability in patients considered to be at increased risk. Genetic testing to determine if a patient is predisposed to poor clopidogrel metabolism ("poor metabolizers") may be considered before starting clopidogrel

therapy in patients believed to be at moderate or high risk for poor outcomes. This might include, among others, patients undergoing elective high-risk PCI procedures (eg, treatment of extensive and/or very complex disease). If such testing identifies a potential poor metabolizer, other therapies, particularly prasugrel for coronary patients, should be considered. With these other therapies, the balance of potential ischemic benefit with the known increased risk of bleeding should be considered either with alternative clopidogrel dosing or newer agents such as prasugrel. In particular, prasugrel is contraindicated in patients with stroke and TIA. For patients with ischemic stroke or TIA, alternatives to clopidogrel include aspirin or the combination of aspirin and extended-release dipyridamole, which are both recommended in the AHA Stroke Council guidelines for secondary prevention of stroke.[66]

6. There are several possible therapeutic options for patients who experience an adverse event while taking clopidogrel in the absence of any concern about medication compliance. Clopidogrel may be switched to prasugrel, which has been found to result in decreased rates of stent thrombosis, and as noted previously, prasugrel is contraindicated for patients with stroke or TIA in patients treated with PCI for ACS, although it has not been tested in randomized trials of patients with stent thrombosis. Alternatively, the physician may make the empiric recommendation to increase the dose of clopidogrel (eg, to 150 mg per day). There are very little data to judge the trade-off of high-dose clopidogrel versus alternative therapies on the risk-benefit ratio of safety (avoidance of bleeding) versus efficacy (prevention of a second recurrence). Functional testing may be performed and may be considered in an attempt to determine if patients are clopidogrel nonresponders. There are several different platelet function tests that can be used to assess the platelet response to clopidogrel, and the clinician should use the method with the greatest reliability and reproducibility at his or her specific facility. For stroke patients, aspirin or the combination of aspirin and extended-release dipyridamole are alternatives, as noted in the preceding text.
7. Higher LDs (600 mg versus 300 mg), double-dose loading (600 mg twice over 2 hours), and higher MDs of clopidogrel (150 mg daily) have been found to improve platelet inhibition and might be considered alternatives for high-risk patients who respond poorly to standard loading and MDs of clopidogrel, although there is uncertainty of the long-term safety and efficacy of this approach. New antiplatelet drugs such as prasugrel and, if FDA approved, ticagrelor are additional alternatives in coronary patients with a known poor response to clopidogrel or in patients at high risk for a poor outcome from potential clopidogrel nonresponsiveness. Their use may obviate the need for additional testing. Other possibilities are adding cilostazol to standard doses of aspirin and clopidogrel[67] or using cilostazol alone.[68–70] However, because platelet inhibition still may not be optimal with these regimens, follow-up platelet function testing might be considered to ensure adequate platelet inhibition. The risk-benefit ratio, in terms of safety and efficacy of each of these alternative strategies, remains to be determined by adequately powered clinical trials.

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References

1. FDA Drug Safety Communication: reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug. Available at: http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm203888.htm. Accessed April 28, 2010.
2. Collet JP, Hulot JS, Pena A, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet. 2009;373:309–17.
3. Giusti B, Gori AM, Marcucci R, et al. Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. Am J Cardiol. 2009;103:806–11.
4. Sibbing D, Stegherr J, Latz W, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. Eur Heart J. 2009;30:916–22.
5. Stockbridge N. FDA Letter of Approval: May 5, 2009, NDA 20-839/S-040. U.S. Food and Drug Administration. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/appletter/2009/020839s040ltr.pdf. Accessed April 29, 2010.
6. Southworth MR. FDA Letter of Approval: November 12, 2009, NDA 20-839/S-044. U.S. Food and Drug Administration. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/appletter/2009/020839s044ltr.pdf. Accessed April 29, 2010.
7. Table of Valid Genomic Biomarkers in the Context of Approved Drug Labels. Available at: http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm. Accessed May 18, 2010.
8. Tegretol (carbamazepine USP) Label. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/016608s101,018281s048lbl.pdf. Accessed May 17, 2010.
9. Highlights of Prescribing Information: Ziagen (abacavir sulfate) Tablets and Oral Solution. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/020977s019,020978s022lbl.pdf. Accessed May 17, 2010.
10. Coumadin (Warfarin) Tablets and Crystalline for Injection Label. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/009218s108lbl.pdf. Accessed May 17, 2010.

10a. Code of Federal Regulations, 21 CFR §201.57 (e). (2009).

11. Murphy S, Roberts R. "Black box" 101: how the Food and Drug Administration evaluates, communicates, and manages drug benefit/risk. J Allergy Clin Immunol. 2006;117:34–9.
12. Gurbel PA, Bliden KP, Hiatt BL, et al. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation. 2003;107:2908–13.
13. Sofi F, Marcucci R, Gori AM, et al. Clopidogrel non-responsiveness and risk of cardiovascular morbidity: an updated meta-analysis. Thromb Haemost. 2010;103:841–8.
14. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation. 2004;109:3171–5.

15. Shuldiner AR, O'Connell JR, Bliden KP, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA. 2009;302:849–57.
16. Mega JL, Close SL, Wiviott SD, et al. Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. Circulation. 2009;119:2553–60.
17. Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med. 2009;360:354–62.
18. Luo HR, Poland RE, Lin KM, et al. Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: a cross-ethnic comparative study. Clin Pharmacol Ther. 2006;80:33–40.
19. Man M, Farmen M, Dumaual C, et al. Genetic variation in metabolizing enzyme and transporter genes: comprehensive assessment in 3 major East Asian subpopulations with comparison to Caucasians and Africans. J Clin Pharmacol. 2010: published online before print February 19, 2010, doi: 10.1177/0091270009353161. Accessed June 16, 2010.
20. Sofi F, Giusti B, Marcucci R, et al. Cytochrome P450 2C19*2 polymorphism and cardiovascular recurrences in patients taking clopidogrel: a meta-analysis. Pharmacogenomics J. 2010: published online before print March 30, 2010, doi: 10.1038/tpj.2010.21. Accessed June 16, 2010.
21. Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. J Am Coll Cardiol. 2008;51:1925–34.
22. Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med. 2009;360:363–75.
23. Varenhorst C, James S, Erlinge D, et al. Genetic variation of CYP2C19 affects both pharmacokinetic and pharmacodynamic responses to clopidogrel but not prasugrel in aspirin-treated patients with coronary artery disease. Eur Heart J. 2009;30:1744–52.
24. James S, Akerblom A, Cannon CP, et al. Comparison of ticagrelor, the first reversible oral P2Y(12) receptor antagonist, with clopidogrel in patients with acute coronary syndromes: rationale, design, and baseline characteristics of the PLATelet inhibition and patient Outcomes (PLATO) trial. Am Heart J. 2009;157:599–605.
25. Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2009;361:1045–57.
26. Gurbel PA, Bliden KP, Antonino MJ, et al. The effect of elinogrel on high platelet reactivity during dual antiplatelet therapy and the relation to CYP2C19*2 genotype: first experience in patients. J Thromb Haemost. 2010;8:43–53.
27. Marin F, Gonzalez-Conejero R, Capranzano P, et al. Pharmacogenetics in cardiovascular antithrombotic therapy. J Am Coll Cardiol. 2009;54:1041–57.
28. Taubert D, von Beckerath N, Grimberg G, et al. Impact of P-glycoprotein on clopidogrel absorption. Clin Pharmacol Ther. 2006;80:486–501.
29. Mega JL, Close SL, Wiviott SD, et al. ABCB1 genetic variants, pharmacodynamic response, and cardiovascular outcomes following treatment with clopidogrel and prasugrel. J Am Coll Cardiol. 2010;55:A113. Abstract.
30. Suh JW, Koo BK, Zhang SY, et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. CMAJ. 2006;174:1715–22.
31. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Lack of association between the P2Y12 receptor gene polymorphism and platelet response to clopidogrel in patients with coronary artery disease. Thromb Res. 2005;116:491–7.
32. Kastrati A, von BN, Joost A, et al. Loading with 600 mg clopidogrel in patients with coronary artery disease with and without chronic clopidogrel therapy. Circulation. 2004;110:1916–9.
33. von Beckerath N, Taubert D, Pogatsa-Murray G, et al. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) trial. Circulation. 2005;112:2946–50.
34. von Beckerath N, Kastrati A, Wieczorek A, et al. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. Eur Heart J. 2007;28:1814–9.
35. Angiolillo DJ, Shoemaker SB, Desai B, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. Circulation. 2007;115:708–16.
36. Footana P, Senouf D, Mach F. Biological effect of increased maintenance dose of clopidogrel in cardiovascular outpatients and influence of the cytochrome P450 2C19*2 allele on clopidogrel responsiveness. Thromb Res. 2008;121:463–8.
37. Gladding P, Webster M, Zeng I, et al. The antiplatelet effect of higher loading and maintenance dose regimens of clopidogrel: the PRINC (Plavix Response in Coronary Intervention) trial. J Am Coll Cardiol Intv. 2008;1:612–9.
38. Alcil B, Jacquemin L, De Poli F, et al. Clopidogrel 150 mg/day to overcome low responsiveness in patients undergoing elective percutaneous coronary intervention: results from the VASP-02 (Vasodilator-Stimulated Phosphoprotein-02) randomized study. J Am Coll Cardiol Intv. 2008;1:631–8.
39. Gurbel PA, Bliden KP, Zaman KA, et al. Clopidogrel loading with eptifibatide to arrest the reactivity of platelets: results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEAR PLATELETS) study. Circulation. 2005;111:1153–9.
40. Price MJ, Coleman JL, Steinhubl SR, et al. Onset and offset of platelet inhibition after high-dose clopidogrel loading and standard daily therapy measured by a point-of-care assay in healthy volunteers. Am J Cardiol. 2006;98:681–4.
41. Montalescot G, Sideris G, Meuleman C, et al. A randomized comparison of high clopidogrel loading doses in patients with non–ST-segment elevation acute coronary syndromes: the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) trial. J Am Coll Cardiol. 2006;48:931–8.
41a. Mehta SR. Talk presented at the Hotline Session European Society of Cardiology; August 2009, Barcelona, Spain.
42. DiSciascio G, Patti G, Pasceri V, et al. Clopidogrel reloading in patients undergoing percutaneous coronary intervention on chronic clopidogrel therapy: results of the ARMYDA-4 RELOAD (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty) randomized trial. Eur Heart J. 2010;31:1317–23.
43. Dangas G, Mehran R, Guagliumi G, et al. Role of clopidogrel loading dose in patients with ST-segment elevation myocardial infarction undergoing primary angioplasty: results from the HORIZONS-AMI (Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction) trial. J Am Coll Cardiol. 2009;54:1438–46.
44. Gurbel PA, Tantry US. Combination antithrombotic therapies. Circulation. 2010;121:569–83.
45. Jeong YH, Lee SW, Choi BR, et al. Randomized comparison of adjunctive cilostazol versus high maintenance dose clopidogrel in patients with high post-treatment platelet reactivity: results of the ACCEL-RESISTANCE (Adjunctive Cilostazol Versus High Maintenance Dose Clopidogrel in Patients With Clopidogrel Resistance) randomized study. J Am Coll Cardiol. 2009;53:1101–9.
46. Jeong YH, Hwang JY, Kim IS, et al. Adding cilostazol to dual antiplatelet therapy achieves greater platelet inhibition than high maintenance dose clopidogrel in patients with acute myocardial infarction: results of the adjunctive cilostazol versus high maintenance dose clopidogrel in patients with AMI (ACCEL-AMI) study. Circ Cardiovasc Interv. 2010;3:17–26.
47. Han Y, Li Y, Wang S, et al. Cilostazol in addition to aspirin and clopidogrel improves long-term outcomes after percutaneous coronary intervention in patients with acute coronary syndromes: a randomized, controlled study. Am Heart J. 2009;157:733–9.
47a. H.S. Kim. Talk presented at the American College of Cardiology Annual Scientific Session/i2 Summit; March 2010, Atlanta, GA.
48. Gajos G, Rostoff P, Undas A, et al. Effects of polyunsaturated omega-3 fatty acids on responsiveness to dual antiplatelet therapy in patients undergoing percutaneous coronary intervention: the OMEGA-PCI (OMEGA-3 fatty acids after PCI to modify responsiveness to dual antiplatelet therapy) study. J Am Coll Cardiol. 2010;55:1671–8.
49. Cuisset T, Frere C, Quilici J, et al. Glycoprotein IIb/IIIa inhibitors improve outcome after coronary stenting in clopidogrel nonresponders: a prospective, randomized study. J Am Coll Cardiol Intv. 2008;1:649–53.
50. Valgimigli M, Campo G, de Cesare N, et al. Intensifying platelet inhibition with tirofiban in poor responders to aspirin, clopidogrel, or both agents undergoing elective coronary intervention: results from the double-blind, prospective, randomized Tailoring Treatment with Tirofi-

ban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel study. Circulation. 2009;119:3215–22.
51. Wiviott SD, Trenk D, Frelinger AL, et al. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. Circulation. 2007;116:2923–32.
52. Michelson AD, Frelinger AL III, Braunwald E, et al. Pharmacodynamic assessment of platelet inhibition by prasugrel vs. clopidogrel in the TRITON–TIMI 38 trial. Eur Heart J. 2009;30:1753–63.
53. Brandt JT, Payne CD, Wiviott SD, et al. A comparison of prasugrel and clopidogrel loading doses on platelet function: magnitude of platelet inhibition is related to active metabolite formation. Am Heart J. 2007;153:e9–16.
54. Jernberg T, Payne CD, Winters KJ, et al. Prasugrel achieves greater inhibition of platelet aggregation and a lower rate of non-responders compared with clopidogrel in aspirin-treated patients with stable coronary artery disease. Eur Heart J. 2006;27:1166–73.
55. Wallentin L, Varenhorst C, James S, et al. Prasugrel achieves greater and faster P2Y12receptor-mediated platelet inhibition than clopidogrel due to more efficient generation of its active metabolite in aspirin-treated patients with coronary artery disease. Eur Heart J. 2008;29:21–30.
56. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2007;357:2001–15.
57. Cannon CP, Harrington RA, James S, et al. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. Lancet. 2010;375:283–93.
58. Gurbel PA, Bliden KP, Butler K, et al. Response to ticagrelor in clopidogrel nonresponders and responders and effect of switching therapies: the RESPOND study. Circulation. 2010;121:1188–99.
58a. Mega JL. Clopidogrel and genetic variance. Presentation given at: 2nd Annual Critical Markers of Disease Canadian Biomarkers and Surrogate Endpoints Meeting; May 17, 2010; Turney's Pasture, Ottawa, Ontario. http://www.cmod.org/images/CMOD_Presentations_5-28-10/Mega%20CMOD%20Ottawa%20May%2017%202010.pdf.
59. Bonello L, Camoin-Jau L, Arques S, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. J Am Coll Cardiol. 2008;51:1404–11.
60. Bonello L, Camoin-Jau L, Armero S, et al. Tailored clopidogrel loading dose according to platelet reactivity monitoring to prevent acute and subacute stent thrombosis. Am J Cardiol. 2009;103:5–10.
61. Pena A, Collet JP, Hulot JS, et al. Can we override clopidogrel resistance? Circulation. 2009;119:2854–7.
62. Gurbel PA, Bliden KP, Butler K, et al. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. Circulation. 2009;120:2577–85.
63. Price MJ, Endemann S, Gollapudi RR, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. Eur Heart J. 2008;29:992–1000.
64. Marcucci R, Gori AM, Paniccia R, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. Circulation. 2009;119:237–42.
65. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. JAMA. 2010;303:754–62.
66. Sacco RL, Adams R, Albers G, et al. Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack: a statement for healthcare professionals from the American Heart Association/American Stroke Association Council on Stroke: co-sponsored by the Council on Cardiovascular Radiology and Intervention: the American Academy of Neurology affirms the value of this guideline. Stroke. 2006;37:577–617.
67. Jennings DL, Kalus JS. Addition of cilostazol to aspirin and a thienopyridine for prevention of restenosis after coronary artery stenting: a meta-analysis. J Clin Pharmacol. 2010;50:415–21.
68. Kwon SU, Cho YJ, Koo JS, et al. Cilostazol prevents the progression of the symptomatic intracranial arterial stenosis: the multicenter double-blind placebo-controlled trial of cilostazol in symptomatic intracranial arterial stenosis. Stroke. 2005;36:782–6.
69. Huang Y, Cheng Y, Wu J, et al. Cilostazol as an alternative to aspirin after ischaemic stroke: a randomised, double-blind, pilot study. Lancet Neurol. 2008;7:494–9.
70. Gotoh F, Toghi H, Hirai S, et al. Cilostazol stroke prevention study: a placebo-controlled double-blind trial for secondary prevention of cerebral infarction. J Stroke Cerebrovasc Dis. 2000;9:147–57.

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Appendix 1. Author Relationships With Industry and Others—ACCF/AHA Clopidogrel Clinical Alert: Approaches to the FDA "Boxed Warning"

Name	Employment	Consultant	Speaker	Ownership/ Partnership/ Principal	Research	Institutional, Organizational, or Other Financial Benefit	Expert Witness
David R. Holmes, Jr, Chair	Consultant—Cardiovascular Diseases, Mayo Clinic	None	None	None	None	None	None
Gregory J. Dehmer	Scott & White Healthcare, Professor of Medicine—Texas A&M College of Medicine	None	None	None	None	None	None
Sanjay Kaul	Director, Cardiology Fellowship Training Program—Cedars-Sinai Heart Institute Professor of Medicine—Cedars-Sinai Medical Center, and David Geffen School of Medicine at UCLA	None	None	• Johnson & Johnson	None	None	None
Dana Leifer	Associate Attending Neurologist—New York Presbyterian Hospital Associate Professor of Neurology—Weill Cornell Medical College	None	None	None	• Medtronic, CRYSTAL AF*	None	None
Patrick T. O'Gara	Associate Professor of Medicine—Brigham and Women's Hospital Cardiovascular Medicine	None	None	None	None	None	None
C. Michael Stein	Dan May Professor of Medicine and Pharmacology—Vanderbilt Medical School Division of Clinical Pharmacology	None	None	None	None	None	None

This table represents the relationships of committee members with industry and other entities that were reported by authors to be relevant to this document. These relationships were reviewed and updated in conjunction with all meetings and/or conference calls of the writing committee during the document development process. The table does not necessarily reflect relationships with industry at the time of publication. A person is deemed to have a significant interest in a business if the interest represents ownership of 5% or more of the voting stock or share of the business entity, or ownership of $10 000 or more of the fair market value of the business entity, or if funds received by the person from the business entity exceed 5% of the person's gross income for the previous year. A relationship is considered to be modest if it is less than significant under the preceding definition. Relationships in this table are modest unless otherwise noted.

*No financial relationship.

Appendix 2. Reviewer Relationships With Industry and Other Entities—ACCF/AHA Clopidogrel Clinical Alert: Approaches to the FDA "Boxed Warning"

Peer Reviewer	Representation	Consultant	Speaker	Ownership/ Partnership/ Principal	Research	Institutional, Organizational, or Other Financial Benefit	Expert Witness
Deepak L. Bhatt	Official Reviewer—ACCF Task Force on Clinical Expert Consensus Documents	• Duke Clinical Research Institute	None	None	• AstraZeneca* • Bristol-Myers Squibb* • Cogentus • Eisai* • PLx Pharma • Sanofi-Aventis* • Takeda • The Medicines Company*	None	• Testimony for defendant on antithrombotic therapy in cardiovascular medicine, 2005
Paul Gurbel	Official Reviewer—American Heart Association	• Accumetrics* • AstraZeneca* • Bayer* • Medtronic* • PlaCor • Pozen • Schering-Plough*	None	None	• Medtronic* • Portola* • Pozen* • Sanofi-Aventis*	None	None

(Continued)

Appendix 2. Continued

Peer Reviewer	Representation	Consultant	Speaker	Ownership/ Partnership/ Principal	Research	Institutional, Organizational, or Other Financial Benefit	Expert Witness
Julie Johnson	Official Reviewer—American Heart Association	• Medco	None	None	• National Institutes of Health*	None	None
Richard J. Kovacs	Official Reviewer—ACCF Board of Trustees	• Abbott Laboratories • Biomedical Systems • Cook–Med Institute* • ECG Scanning Services* • Eli Lilly* • Endocyte • Essentialis • XenoPort	None	None	None	None	None
Michael E. Ring	Official Reviewer—ACCF Board of Governors	None	None	None	None	None	None
Robert Lee Page II	Official Reviewer—American Heart Association	None	None	None	None	None	None
Dominick J. Angeliillo	Organizational Reviewer—Society for Cardiovascular Angiography and Interventions	• Accumetrics • Arena • Astra-Zeneca • Bristol-Myers Squibb* • Daiichi Sankyo* • Eli Lilly* • Merck • Novartis • Portola • Sanofi-Aventis*	• Bristol-Myers Squibb* • Daiichi Sankyo* • Eli Lilly* • Sanofi-Aventis*	None	• Accumetrics* • Astra-Zeneca* • Daiichi Sankyo* • Eisai* • Eli Lilly* • GlaxoSmithKline* • Johnson & Johnson* • Portola* • Schering-Plough* • The Medicines Company*	None	None
Thomas M. Beaver	Organizational Reviewer—Society of Thoracic Surgeons	• Pfizer	None	None	None	None	None
Doug Campos-Outcalt	Official Reviewer—American Association of Family Physicians	None	None	None	None	None	None
Donald E. Casey, Jr.	Official Reviewer—American College of Physicians	None	None	None	None	None	None
Matthew J. Price	Organizational Reviewer—Society for Cardiovascular Angiography and Interventions	• Accumetrics* • AstraZeneca • Bristol-Myers Squibb/ Sanofi-Aventis • DSV/Lilly	• DSV/ Lilly	None	• Bristol-Myers Squibb/ Sanofi*	None	None
Craig H. Selzman	Organizational Reviewer—Society of Thoracic Surgeons	None	None	None	None	None	None
Jeffrey L. Anderson	Content Reviewer—ACCF UA Guideline	None	None	None	None	None	None
Eric R. Bates	Content Reviewer—ACCF PCI Guideline	• Bristol-Myers Squibb • Daiichi Sankyo • Eli Lilly • Momenta • Novartis • Sanofi-Aventis • Takeda	None	None	None	None	None
John G. Byrne	Content Reviewer—ACC Surgeon Scientific Council	None	None	None	None	None	None

(*Continued*)

Appendix 2. Continued

Peer Reviewer	Representation	Consultant	Speaker	Ownership/ Partnership/ Principal	Research	Institutional, Organizational, or Other Financial Benefit	Expert Witness
Victor A. Ferrari	Content Reviewer—ACCF Task Force on Clinical Expert Consensus Documents	None	None	None	None	None	None
Federico Gentile	Content Reviewer—ACCF Task Force on Clinical Expert Consensus Documents	None	None	None	None	None	None
Jonathan L. Halperin	Content Reviewer—ACCF Extracranial and Vertebral Artery Disease Guideline	• Astellas • Bayer • Biotronik* • Boehringer Ingelheim* • Daiichi Sankyo Pharma • Johnson & Johnson* • Portola • Sanofi-Aventis*	None	None	None	None	None
Robert A. Harrington	Content Reviewer—ACCF Task Force on Clinical Expert Consensus Documents	• AstraZeneca* • Baxter • CSL Behring • Eli Lilly • Luitpoid • Merck • Novartis • Otsuka Maryland Research Institute • Regado • Schering-Plough* • Sanofi-Aventis • The Medicines Company	None	None	• AstraZeneca • Baxter • Bristol-Myers Squibb* • GlaxoSmithKline* • Merck* • Portola* • Schering-Plough* • The Medicines Company	None	None
L. David Hillis	Content Reviewer—ACCF CABG Guideline	None	None	None	None	None	None
Frederick G. Kushner	Content Reviewer—ACCF STEMI Guideline	• FDA	None	• Bristol-Myers Squibb • Pfizer • Merck • Roche Holding*	• Daiichi-Sankyo • Hoffmann La Roche • Novartis	None	None
Gordon F. Tomaselli	Content Reviewer—ACCF Proton Pump Inhibitor Expert Consensus Document	None	None	None	None	None	None

This table represents the relevant relationships with industry and other entities that were disclosed at the time of peer review. It does not necessarily reflect relationships with industry at the time of publication. A person is deemed to have a significant interest in a business if the interest represents ownership of 5% or more of the voting stock or share of the business entity, or ownership of $10 000 or more of the fair market value of the business entity, or if funds received by the person from the business entity exceed 5% of the person's gross income for the previous year. A relationship is considered to be modest if it is less than significant under the preceding definition. Relationships in this table are modest unless otherwise noted.

*Significant (greater than $10 000) relationship.

CABG indicates coronary artery bypass grafting; PCI, percutaneous coronary intervention; STEMI, ST segment elevation myocardial infarction; and UA, unstable angina.

Appendix 3. FDA Drug Safety Communication: Reduced Effectiveness of Clopidogrel in Patients Who Are Poor Metabolizers of the Drug

Safety Announcement

[03-12-2010] The U.S. Food and Drug Administration (FDA) has added a *Boxed Warning* to [sanofi-aventis, Bridgewater, NJ] for Plavix, the antiblood clotting medication. The *Boxed Warning* is about patients who do not effectively metabolize the drug (ie, "poor metabolizers") and therefore may not receive the full benefits of the drug.

The *Boxed Warning* in the drug label will include information to:

- Warn about reduced effectiveness in patients who are poor metabolizers of Plavix. Poor metabolizers do not effectively convert Plavix to its active form in the body.
- Inform healthcare professionals that tests are available to identify genetic differences in CYP2C19 function.
- Advise healthcare professionals to consider use of other antiplatelet medications or alternative dosing strategies for Plavix in patients identified as poor metabolizers.

Plavix is given to reduce the risk of heart attack, unstable angina, stroke, and cardiovascular death in patients with cardiovascular disease. Plavix works by decreasing the activity of blood cells called platelets, making platelets less likely to form blood clots.

For Plavix to work, enzymes in the liver (particularly CYP2C19) must convert (metabolize) the drug to its active form. Patients who are poor metabolizers of the drug do not effectively convert Plavix to its active form. In these patients, Plavix has less effect on platelets, and therefore less ability to prevent heart attack, stroke, and cardiovascular death. It is estimated that 2% to 14% of the population are poor metabolizers; the rate varies based on racial background.

Healthcare professionals should be aware that a subgroup of patients are poor metabolizers and do not metabolize Plavix effectively; this can result in reduced effectiveness of Plavix. Healthcare professionals should consider use of other antiplatelet medications or alternative dosing strategies for Plavix in these patients.

Patients should not stop taking Plavix unless told to do so by their healthcare professional. They should talk with their healthcare professional if they have any concerns about Plavix, or to find out if they should be tested for being a poor metabolizer.

In May 2009, the FDA added information about poor metabolizers of Plavix to the drug label. However, based on additional data reviewed by the agency (see Data Summary in the following text), the *Boxed Warning* is now being added to highlight the reduced effectiveness of Plavix in these patients and to recommend that healthcare professionals consider use of other antiplatelet medications or alternative dosing strategies for Plavix in patients identified as poor metabolizers.

Additional Information for Patients

Patients currently taking Plavix should:

- Be aware that some patients do not convert Plavix to its active form as well as other patients. These patients may not get the same benefit from Plavix and are known as poor metabolizers.
- Do not stop taking Plavix unless told to do so by their healthcare professional.
- Talk with their healthcare professional if they have any concerns about Plavix.
- Talk with their healthcare professional to see if testing to determine their metabolizer status is appropriate.

Additional Information for Healthcare Professionals

The FDA recommends that healthcare professionals should:

- Be aware that some patients may be poor metabolizers of Plavix. They do not effectively convert Plavix to its active form because of low CYP 2C19 activity. The effectiveness of Plavix as a preventive therapy is reduced in these patients.
- Be aware that tests are available to determine patients CYP2C19 status.
- Consider use of other antiplatelet medications or alternative dosing strategies for Plavix in patients who have been identified as poor metabolizers.
- Be aware that although a higher dose regimen (600 mg loading dose followed by 150 mg once daily) in poor metabolizers increases antiplatelet response, an appropriate dose regimen for poor metabolizers has not been established in a clinical outcome trial.
- Review the newly approved Plavix drug label for complete information on the use of Plavix

Data Summary

The liver enzyme CYP2C19 is primarily responsible for the formation of the active metabolite of Plavix. Pharmacokinetic and antiplatelet tests of the active metabolite of Plavix show that the drug levels and antiplatelet effects differ depending on the genotype of the CYP2C19 enzyme. The following represent the different alleles of CYP2C19 that make up a patient's genotype:

- The CYP2C19*1 allele has fully functional metabolism of Plavix.
- The CYP2C19*2 and *3 alleles have no functional metabolism of Plavix. These 2 alleles account for mostof the reduced function alleles in patients of [European] (85%) and Asian (99%) descent classified as poor metabolizers.
- The CYP2C19*4, *5, *6, *7, and *8 and other alleles may be associated with absent or reduced metabolism of Plavix, but are less frequent than the CYP2C19*2 and *3 alleles.
- A patient with 2 loss-of-function alleles (as defined in the preceding text) will have poor metabolizer status.

The pharmacokinetic and antiplatelet responses to Plavix were evaluated in a crossover trial in 40 healthy subjects. Ten subjects in each of the 4 CYP2C19 metabolizer groups (ultrarapid, extensive, intermediate, and poor) were randomized to 2

treatment regimens: a 300 mg loading dose followed by 75 mg per day, or a 600 mg loading dose followed by 150 mg per day, each for a total of 5 days. After a washout period, subjects were crossed over to the alternate treatment. Decreased active metabolite exposure and increased platelet aggregation were observed in the poor metabolizers compared with that seen in the other groups. When poor metabolizers received the 600 mg loading dose followed by 150 mg daily, active metabolite exposure and antiplatelet response were greater than with the 300 mg/75 mg regimen. Healthcare professionals should note that an appropriate dose regimen for patients who are poor metabolizers has not been established in clinical outcome trials.[1]

More Information

Related Information

- FDA Drug Safety Communication: Reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug. Release date: 3/12/2010
- Public Health Advisory: Updated Safety Information about a drug interaction between clopidogrel bisulfate (marketed as Plavix) and omeprazole (marketed as Prilosec and Prilosec OTC). Release date: 11/17/2009
- Information for Healthcare Professionals: Update to the labeling of Clopidogrel Bisulfate (marketed as Plavix) to alert healthcare professionals about a drug interaction with omeprazole (marketed as Prilosec and Prilosec OTC). Release date: 11/17/2009
- Follow-Up to the January 26, 2009, Early Communication about an Ongoing Safety Review of Clopidogrel Bisulfate (marketed as Plavix) and Omeprazole (marketed as Prilosec and Prilosec OTC). Release date: 11/17/2009
- Early Communication about an Ongoing Safety Review of clopidogrel bisulfate (marketed as Plavix). Release date: 1/26/2009
- Clopidogrel (marketed as Plavix) and Omeprazole (marketed as Prilosec)–Drug Interaction. Release date: 11/17/2009

Exhibit G

Circulation
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Responding to the Clopidogrel Warning by the US Food and Drug Administration: Real Life Is Complicated

Dan M. Roden and Alan R. Shuldiner

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Responding to the Clopidogrel Warning by the US Food and Drug Administration

Real Life Is Complicated

Dan M. Roden, MD; Alan R. Shuldiner, MD

A sine qua non for drug approval by the US Food and Drug Administration (FDA) is demonstrated efficacy in populations of patients. However, it is virtually axiomatic that individuals vary in their responses to drugs. Work over decades has built a knowledge base that describes the role of genetic variation as a modulator of both drug efficacy and rare adverse drug effects.[1] This increasing understanding of the role of genetics in variable drug responses led the FDA in 2007 to begin to incorporate pharmacogenetic information in drug labels.[2]

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In some cases, available data have allowed drug labeling to include specific and highly directive advice. For example, empirical studies, followed by a large randomized clinical trial, demonstrated that preprescription genotyping to avoid the antiretroviral agent abacavir in patients carrying the human leukocyte antigen B*5701 variant can strikingly reduce, if not eliminate, the risk of drug-related severe skin reactions.[3,4] The FDA label now carries the unambiguous warning stating that such testing should be done and the drug not prescribed in patients with the variant. However, it is likely that single genetic variants with such large effects and predictive value on drug response or adverse effects are more often the exception than the rule; rather, a few or many genetic variants, each with relatively modest effect, contribute to a continuum of drug response in the treated population. Defining the clinical utility of such genetic variants poses important challenges to how pharmacogenetic information may be incorporated into practice. The widespread use of clopidogrel, with its well-documented large interindividual variation in response to the drug and the emerging understanding of the genetics of that variability, is the latest example of such a challenge.

What Is Known

Remarkably, when clopidogrel was approved in 1997, its mechanism of action was not known. Great interindividual variability in response was recognized soon after,[5] and since then, we have learned that clopidogrel must first be converted to an active metabolite, which then binds and irreversibly inhibits P2Y12 (ADP) receptors on platelets to exert its antiplatelet effect.[6] Studies indicate that this bioactivation step is largely but not exclusively dependent on the activity of a specific hepatic cytochrome P450 enzyme, termed CYP2C19.[7] There are several common variants of the *CYP2C19* gene. The normally functioning allele is termed *1, but the *2 allele, which results in loss of function of the encoded protein, is common across many populations. Homozygotes for the loss of function allele (poor metabolizers) represent 2% to 3% of whites and blacks, and up to 15% to 20% of East Asians; heterozygotes represent 30% to 35% and 40% to 45% of these populations, respectively. When ex vivo measures of platelet aggregation are used to define drug effect, loss of function alleles can be shown to decrease drug action in a gene-dose dependent fashion[7,8]; that is, individuals treated with clopidogrel with the *2/*2 genotype are less responsive than those with the *1/*2 genotype (intermediate metabolizers) who in turn are less responsive than those with the *1/*1 genotype. The surrogate end point of inhibition of platelet aggregation has been partially validated by retrospective examinations of outcomes in patients receiving the drug for clinical indications, in which *2/*2 homozygotes (and possibly also *1/*2 heterozygotes) display increased cardiovascular event rates compared to those with the *1/*1 genotype.[8–10] These recent findings have led to the FDA-mandated black box label for clopidogrel that now alerts physicians and patients of the role of common *CYP2C19* gene variants in mediating the drug's actions.

What Is Uncertain

Despite the dependency of clopidogrel bioactivation on CYP2C19 activity, not all studies show increased cardiovascular events in patients on clopidogrel with the *1/*2 genotype compared to those with *1/*1.[10] In addition, the effect of rarer *CYP2C19* variants that reduce enzyme function (eg, *3 or *5) has not been studied. Emerging data suggest that *CYP2C19*17*, a relatively common allele that results in increased enzyme expression and activity, may be associated with a modest increase in clopidogrel responsiveness.[11,12] However, the *2 and *17 variants are in linkage disequilibrium, so it is not certain that the effects of this variant are independent of that of the *2 variant.[13] Some proton pump

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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inhibitors (PPIs), notably omeprazole,[14] are potent CYP2C19 inhibitors, and omeprazole's reversal of clopidogrel's effect on ex vivo measured platelet function is readily demonstrated. However, there are highly contradictory data on whether coadministration of PPIs and clopidogrel alters cardiovascular event rates.[15–17]

Most importantly, no studies have been published to define a clinical strategy that would exploit this pharmacogenetic information to optimize outcomes with clopidogrel. Thus, for example, although increasing the dose in *2/*2 subjects seems rational, limited available data do not strongly support this strategy.[18] Whereas the FDA's warning does serve to bring the attention of the prescribing community to new data that affects variability in response to drug therapy, the advisory has also generated concern because the practitioner is only offered a series of possible responses, none of which has been tested in any reasonable fashion.

Why Is This So Confusing?

We suggest that one explanation for this confusion arises from differing expectations—in the genetics community, among clinicians, and perhaps among regulators—over the contribution of single genetic variants to common human traits. In the genomics community, there is now an emerging consensus that common gene variants explain a smaller proportion of the heritability of common diseases than had been anticipated.[19] Pharmacogenetics "hype" has promulgated a vision that knowing one or a few genotypes might allow a clear distinction between responders and nonresponders or help identify those likely to suffer catastrophic side effects. This can happen—abacavir is one example—but the reality is that biology is often much more complicated than a few arrows on a simple linear drug response pathway: clopidogrel → bioactivation (by a single gene product) → effect.

In the case of clopidogrel, we do have data: For example, a large study in the Amish, a group with extensive family relationships, showed that the genetic component of variability in the extent to which clopidogrel inhibits ADP-triggered platelet aggregation was ≈70%.[20] A genome-wide association study identified the *CYP2C19* locus as the single most important contributor to this variability, but the contribution of variability was "only" ≈12%. To a clinician, that may sound like a small number, but to a geneticist this is an enormous contribution. Importantly, there were no other strong association signals apparent in the genome-wide association study suggesting that the majority of the genetic variability in clopidogrel response may be due to more modest effects of many other common variants or perhaps rare or other kinds of genetic variants that escaped detection with current genome-wide association study methodology.

This moderate influence of genetic variation in *CYP2C19* may also explain some of the uncertainties over the PPI effect: it is conceivable that an interaction between PPIs and clopidogrel would only be clinically meaningful in individuals with reduced CYP2C19 activity (eg, *1/*2), whereas *1/*1 homozygotes would display sufficient enzyme activity that PPI coadministration would not alter platelet inhibition. This is a hypothesis to be tested, and in any case, as with all drug therapy, it is important to weigh risks and benefits, and a major benefit of PPIs in this setting is prevention of gastrointestinal hemorrhage.[21]

A recurring theme in complex traits, like pharmacogenomics, is that genetic variation does not confer absolutes, but rather alters probabilities of particular outcomes. This necessarily means that while drug responses may be stochastic ("good" or "bad") in an individual, this is rarely the case in a population: event rates in patients receiving effective P2Y12 inhibition are not zero, nor are they 100% in patients not receiving drug, or in those genetically unable to generate active drug. Physicians can be quite adept at considering multiple lines of probabilistic evidence-based data in formulating a treatment plan for a given patient. However, they are now presented with an FDA warning on *CYP2C19* and clopidogrel in the face of a gap in knowledge as to how to incorporate the *CYP2C19* genotype into their clinical decision-making practices.

What Response Might a Clinician Adopt?

The accompanying American College of Cardiology Foundation/American Heart Association Clopidogrel Clinical Alert[22] nicely outlines possible actions by clinicians:

- Do nothing; follow guidelines: This is a default position, and is tenable in the absence of availability of any other data or testing. This may especially be the case in an interregnum (now) between identification of an important predictor of drug response like *CYP2C19* genotype and solid data on how reasonably to respond to it.
- Use platelet function testing as an alternative to genetic testing: Variability in response to clopidogrel is reminiscent of variable warfarin response; here too, there continues to be argument over the utility of preprescription genotyping as an adjunct to international normalized ratio measurements. The best test of platelet function and how this should be deployed in practice is not yet standardized[23,24]; One appealing option is to incorporate both genetic testing and platelet function monitoring into management of P2Y12 inhibitor therapy.[13,24] Initial genetic testing will identify patients at risk for drug failure, whereas intermittent platelet function testing could be viewed as analogous to international normalized ratio measurements for warfarin and allow the clinician to address the variance in drug action even after CYP2C19*2 is factored in.
- Use preprescription genotyping to guide therapy: Because many cardiovascular events occur within the first few hours to days after percutaneous coronary intervention, a rapid turnaround time is essential. The questions here are how and whether to adjust clopidogrel dose or to choose an alternative drug; and in whom: just poor metabolizers (*2/*2 homozygotes) or also in intermediate metabolizers (*1/*2 heterozygotes)? In addition, third-party payers may or may not reimburse for genetic testing without the evidence base to support its efficacy.
- Ignore clopidogrel and prescribe "alternate P2Y12 inhibitors" (ie, prasugrel for now) to all: Prasugrel action does not appear to be affected by *CYP2C19* genotype. In the Trial to Assess Improvement in Therapeutic Outcomes by

Optimizing Platelet Inhibition With Prasugrel—Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38) trial, the drug resulted in fewer cardiovascular events but more bleeding.[25] Thus, use of prasugrel in all patients would preempt *CYP2C19* genetic testing but increase exposure to adverse bleeding complications. To increase the benefit: risk ratio and manage costs, a more individualized approach might be to prescribe clopidogrel in patients without at-risk genotypes and other drugs such as prasugrel in subjects with *CYP2C19* variant genotypes. This option might also be cost effective, with clopidogrel coming off patent and soon to be much less expensive than newer agents. However, as the American College of Cardiology Foundation/American Heart Association Clopidogrel Clinical Alert correctly points out, the evidence base for this option currently does not exist.

It is clear that none of these options are well supported by data and that major issues are unsettled: eg, which platelet function test is best, how to get timely genetic data on which to act, how to act, and the economics of genetic testing versus complications avoided.

Practice Versus Regulation

The drug label is meant to convey important information for drug use and for marketing.[26] Thus, we believe that the FDA has little choice but to inform prescribers of new information that may affect the way in which their patients respond to drugs. To ignore the *CYP2C19* data would be to place the regulatory agency in the unconscionable position of having a label that does not accurately describe the risks and benefits of drug treatment.

The uncertainties over the use of genetic testing in the management of clopidogrel and other drugs, such as warfarin or tamoxifen, reflect impressive progress in pharmacogenetics coupled to uncertainties over how to incorporate that progress into practice. This is the paradox of evidence-based medicine in populations versus individualized medicine. Whereas the "gold standard" for altering practice is the randomized clinical trial, a major challenge remains development of methods to deploy what we know about genomic variation and human traits. The conduct of randomized clinical trials in large unselected populations, most of whom will not carry risk alleles, is inefficient and cost prohibitive. Thus, it will be important to consider novel study designs such as genotype enrichment in populations at high risk for events, and comparative effectiveness study designs incorporating genetics that clearly define treatment options superior to the current standard of care.

Ignoring the newly emerging data on *CYP2C19* genotype and clopidogrel response does not seem to be the best approach. Another way of looking at the tension in this area is to pose the question: "If the genotyping data were readily and simply available at the time of prescribing, should it be used?" Stated this way, the answer would almost certainly be "yes": there seems little downside to at least knowing which patients can take the standard dose of the about-to-be cheaper drug and which need extra thought. This idea, which might easily apply to many drugs, can be posed because of an extraordinarily rapidly evolving genotyping environment: We are 1 to 2 years (at most) away from sub-$1000 whole genome sequencing. This kind of technological development, which raises a myriad of operational, ethical, educational, interpretative, and regulatory challenges,[27] will enable a much broader view of how near-future pharmacogenomic discoveries will be translated into clinical practice.

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References

1. Sangkuhl K, Berlin DS, Altman RB, Klein TE. PharmGKB: understanding the effects of individual genetic variants. *Drug Metab Rev*. 2008;40:539–551.
2. Lesko LJ, Zineh I. DNA, drugs and chariots: on a decade of pharmacogenomics at the US FDA. *Pharmacogenomics*. 2010;11:507–512.
3. Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, Sayer D, Castley A, Mamotte C, Maxwell D, James I, Christiansen FT. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet*. 2002;359:727–732.
4. Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, Jagel-Guedes E, Rugina S, Kozyrev O, Cid JF, Hay P, Nolan D, Hughes S, Hughes A, Ryan S, Fitch N, Thorborn D, Benbow A, the PRED. HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med*. 2008;358:568–579.
5. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation*. 2003;107:2908–2913.
6. Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, Conley PB. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature*. 2001;409:202–207.
7. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, Aiach M, Lechat P, Gaussem P. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood*. 2006;108:2244–2247.
8. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS. Cytochrome P-450 Polymorphisms and Response to Clopidogrel. *N Engl J Med*. 2009;360:354–362.
9. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Beastimon G, Funck-Brentano C, Montalescot G. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet*. 2009;373:309–317.
10. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med*. 2009;360:363–375.
11. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, Morath T, Schomig A, von Beckerath N, Kastrati A. Cytochrome 2C19*17 Allelic Variant, Platelet Aggregation, Bleeding Events, and Stent Thrombosis in Clopidogrel-Treated Patients With Coronary Stent Placement. *Circulation*. 2010;121:512–518.
12. Sibbing D, Gebhard D, Koch W, Braun S, Stegherr J, Morath T, Von BN, Mehilli J, Schomig A, Schuster T, Kastrati A. Isolated and interactive impact of common CYP2C19 genetic variants on the antiplatelet effect of chronic clopidogrel therapy. *J Thromb Haemost*. 2010.
13. Gurbel PA, Tantry US, Shuldiner AR, Kereiakes DJ. Genotyping One Piece of the Puzzle to Personalize Antiplatelet Therapy. *J Am Coll Cardiol*. 2010.

14. Gilard M, Arnaud B, Le GG, Abgrall JF, Boschat J. Influence of omeprazol on the antiplatelet action of clopidogrel associated to aspirin. *J Thromb Haemost*. 2006;6:2508–2509.
15. Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, Rumsfeld JS. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA*. 2009;301:937–944.
16. Juurlink DN, Gomes T, Ko DT, Szmitko PE, Austin PC, Tu JV, Henry DA, Kopp A, Mamdani MM. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ*. 2009;180:713–718.
17. Depta JP, Bhatt DL. Omeprazole and clopidogrel: should clinicians be worried? *Chew Clin J Med*. 2010;77:113–116.
18. Pena A, Collet JP, Hulot JS, Silvain J, Barthelemy O, Beygui F, Funck-Brentano C, Montalescot G. Can we override clopidogrel resistance? *Circulation*. 2009;119:2854–2857.
19. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. Finding the missing heritability of complex diseases. *Nature*. 2009;461:747–753.
20. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009;302:849–857.
21. Ray WA, Murray KT, Griffin MR, Chung CP, Smalley WE, Hall K, Daugherty JR, Kaltenbach LA, Stein CM. Outcomes with concurrent use of clopidogrel and proton-pump inhibitors: a cohort study. *Ann Intern Med*. 2010;152:337–345.
22. Holmes DR, Dehmer GJ, Kaul S, Leifer D, O'Gara PT, Stein CM. ACCF/AHA Clopidogrel Clinical Alert: Approaches to the FDA "Boxed Warning": A Report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the American Heart Association. *Circulation*. 2010;122:537–557.
23. Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. *J Am Coll Cardiol*. 2007;50:1822–1834.
24. Price MJ, Berger PB, Angiolillo DJ, Teirstein PS, Tanguay JF, Kandzari DE, Cannon CP, Topol EJ. Evaluation of individualized clopidogrel therapy after drug-eluting stent implantation in patients with high residual platelet reactivity: design and rationale of the GRAVITAS trial. *Am Heart J*. 2009;157:818–824, 824.
25. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De SS, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2007;357:2001–2015.
26. Murphy S, Roberts R. "Black box" 101: how the Food and Drug Administration evaluates, communicates, and manages drug benefit/risk. *J Allergy Clin Immunol*. 2006;117:34–39.
27. Hamburg MA, Collins FS. The path to personalized medicine. *N Engl J Med*. Published online before print June 15, 2010. doi: 10.1056/NEJMp1006304. Available at: http://content.nejm.org/cgi/content/full/NEJMp1006304v1. Accessed June 15, 2010.

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Exhibit H

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Prasugrel achieves greater inhibition of platelet aggregation and a lower rate of non-responders compared with clopidogrel in aspirin-treated patients with stable coronary artery disease

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Platelets;
Trials

Aims This study was designed to compare the degree of inhibition of platelet aggregation (IPA) of prasugrel with that of clopidogrel in stable aspirin-treated patients with coronary artery disease (CAD).
Methods and results Subjects ($n = 101$) were randomly assigned to the following loading dose (LD) (day 1)/maintenance dose (MD) (days 2–28) combinations: prasugrel, 40 mg/5 mg; 40 mg/7.5 mg; 60 mg/10 mg; 60 mg/15 mg; or clopidogrel, 300 mg/75 mg. Turbidometric platelet aggregation was measured at multiple timepoints during the study. At 4 h after dosing, with 20 μM ADP, both prasugrel LDs achieved significantly higher mean IPA levels (60.6% and 68.4 vs. 30.0%, respectively; all $P < 0.0001$) and lower percentage (3 vs. 52%, $P < 0.0001$) of pharmacodynamic non-responders (defined as IPA <20%) than clopidogrel. Prasugrel 10 and 15 mg MDs achieved consistently higher mean IPA than clopidogrel 75 mg at day 28 (all $P < 0.0001$). At pre-MD on day 28, there were no non-responders in the 10 and 15 mg prasugrel group, compared with 45% in the clopidogrel group ($P = 0.0007$).
Conclusion In this population, prasugrel (40–60 mg LD and 10–15 mg MD) achieves greater IPA and a lower proportion of pharmacodynamic non-responders compared with the approved clopidogrel dosing.

Introduction

Thienopyridine derivatives inhibit platelet aggregation by blocking adenosine diphosphate (ADP)-dependent activation of platelets via the platelet $P2Y_{12}$ receptor.[1] Several studies have documented that a combination of aspirin and clopidogrel reduces both percutaneous coronary intervention related and spontaneous ischaemic events in patients with non-ST-elevation acute coronary syndrome (ACS) and patients undergoing PCI for stable coronary artery disease (CAD).[2,3] Therefore, the addition of clopidogrel has been recommended as standard care in these patients.[4]

However, subacute stent thrombosis still occurs in 1–3% of the patients receiving dual antiplatelet therapy.[5] Recent studies have demonstrated a marked interindividual variability of clopidogrel's capacity to inhibit platelet aggregation with a substantial proportion (11–34%) of the patients considered non-responders to clopidogrel treatment.[6–9] Thus, a more potent and consistent inhibitor of ADP-dependent platelet activation may offer the potential for improved clinical outcomes in ACS and PCI.

Prasugrel (CS-747) is a new thienopyridine derivative that is ~10 times more potent than clopidogrel in preclinical studies.[10] Prasugrel has been evaluated both in healthy individuals and in a recently reported study in patients undergoing elective or urgent PCI in which it was shown to result in low and similar rates of bleeding when compared with clopidogrel.[11]

The primary objective of the current study was to characterize, in aspirin-treated subjects with stable CAD, the degree of inhibition of platelet aggregation (IPA) associated with four dosing regimens of prasugrel compared with the currently approved clopidogrel loading dose (LD) and maintenance dose (MD) regimen.

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Methods

Patients

Two centres in two countries (Sweden and USA) enrolled patients between November 2002 and October 2003. This randomized (with stratification by centre), partially blind, parallel-group study was conducted in adult male and female patients with CAD, aged 40–75 years. Ethical review board approval was obtained for the study and written informed consent was obtained from each subject. Subjects were eligible for enrolment in the study if they had CAD, defined as subjects diagnosed with chronic stable angina, prior history of unstable angina or acute myocardial infarction, previous coronary revascularization or CAD in at least one coronary vessel at angiography; peripheral artery occlusive disease (intermittent claudication, ankle-brachial index <0.9, or previous peripheral vascular intervention); or a documented previous history of cerebrovascular disease, including ischaemic stroke or history of a previous transient ischaemic attack.

Subjects were excluded from the study if they met any of the following criteria: ACS or PCI within 30 days, peripheral artery occlusive disease within 30 days of hospitalization or requiring previous amputation, history or presence of bleeding disorder, and history of recent surgery or severe trauma. Subjects were also excluded if there was evidence of active hepatic disease, uncontrolled hypertension, arrhythmia, or severe congestive heart failure.

Subjects were also excluded if they had taken thienopyridines, antiplatelet agents (other than aspirin), inhibitors (ciprofloxacin, clarithromycin, erythromycin, fluconazole, fluvoxamine, itraconazole, ketoconazole), or inducers (barbiturates, carbamazepine, phenytoin, rifampicin) of cytochrome P4503A4. In addition, proton pump inhibitors and H_2 receptor antagonists were discontinued prior to the run-in period.

Study design

All subjects received enteric-coated aspirin (325 mg/day, Ecotrin®, GlaxoSmithKline) during a 7-day, open-label, run-in period and throughout the treatment period. After the run-in period, subjects were randomized to LD of study drug on day 1 and MD for 27 days. For logistical reasons, the patients were followed during dosing for a range of 26–32 days. A final study visit was scheduled between 7 and 14 days after the last MD. Prasugrel, supplied as the 2.5, 5, and 10 mg tablets of the base formulation, was manufactured by Sankyo Product Development Laboratories, Shinagawa-ku, Tokyo, Japan. Clopidogrel (Plavix®, Sanofi-Synthelabo) was supplied as 75 mg tablets available commercially. Subjects were randomly assigned to one of five dosing regimens for the treatment period: (i) prasugrel 40 mg LD/5 mg MD; (ii) prasugrel 40 mg LD/7.5 mg MD; (iii) prasugrel 60 mg LD/10 mg MD; (iv) prasugrel 60 mg LD/15 mg MD); or (v) clopidogrel 300 mg LD/75 mg MD. The present study was double blind with respect to the prasugrel dose administered, while both aspirin and clopidogrel were dosed in an open-label manner.

Pharmacodynamic measurements

Venous blood samples of ~15 mL were collected in one-tenth volume of 3.8% sodium citrate at the following timepoints: (i) visit 1 (day 1)—pre-dose (duplicate samples), 2, 4, and 6 h post-dose; (ii) visit 2 (day 7–14)—two post-dose samples collected on the same day at least 1 h apart; (iii) visit 3 (day 26–32)—samples collected pre-dose, 2, 4, and 6 h post-dose.

All laboratory personnel conducting the platelet aggregation studies were blinded as to patient treatment. Platelet-rich and platelet-poor plasma were prepared by differential centrifugation at room temperature. There was no adjustment of platelet count performed. Platelet aggregation studies were completed within 3 h of sample collection. Turbidometric platelet aggregation was performed using platelet-rich plasma, with 0% light transmittance set with subject platelet-rich plasma and 100% transmittance set with subject platelet-poor plasma. The aggregometers used were as follows: in the US, a Bio-Data Model PAP-4; in Sweden a Chronolog 490. Agonists used at each site were from the same source and prepared identically. Platelet aggregation was allowed to proceed for 8 min following addition of the agonist (5 or 20 μM ADP). The maximal platelet aggregation (MPA_t) response during that time was recorded and used for data analysis. IPA was calculated using the following formula: $\%IPA = [(MPA_0 - MPA_t)/MPA_0] \times 100$, where MPA_0 is the MPA at baseline on aspirin alone and MPA_t = MPA at time t on study drug plus aspirin.

Adverse events

Laboratory tests were performed at screening, prior to the first dose of study drug (day −1, day 1, or the run-in visit) and on visits 2 and 3. All unexpected signs and symptoms were recorded throughout the treatment period. Physical examinations were performed at screening and at the post-study visit.

Statistical analysis of platelet aggregation data

IPA data were analysed using a linear mixed-effect model with baseline MPA as a covariate, with fixed effects for dosing regimen, time since first dosing, study site, and for the interactions between dosing regimen and time since first dosing and respectively, between dosing regimen and site as fixed effects, and finally with subject as a random effect. The model allowed intersubject and intrasubject variabilities to be different across the treatment groups and time since first dosing. This analysis was implemented using the SAS MIXED procedure (SAS Institute Inc., Cary, NC, USA, version 8.2).

The primary comparison of interest was between the four prasugrel MD groups and the clopidogrel MD group on day 28 at pre-dose. A second comparison of interest was between the two prasugrel LD groups and the clopidogrel LD group on day 1 at 4 h post-dose. Dunnett's adjustment for multiple comparisons to one control (clopidogrel) was used in both cases. For other comparisons, an overall test was run first, and this test being significant, individual tests were then run between each pair of treatments. All statistical tests performed were two-sided and carried out at the 0.05 significance level.

Statistical analysis of pharmacodynamic non-responders

In order to further characterize the effect of prasugrel and clopidogrel on IPA, the percentage of pharmacodynamic non-responders in each treatment group was analysed. For this analysis, a thienopyridine non-responder on aspirin was defined by IPA criteria as an individual not achieving ≥20% IPA to 20 μM ADP by 4 h after an LD or not maintaining ≥20% IPA at subsequent pre-dose timepoints during MD administration. With 5 μM ADP as the agonist, the criterion defining a non-responder was not maintaining ≥25% IPA. This definition was derived from a model based on data acquired from previous investigations of clopidogrel in healthy human subjects, including intrasubject and intersubject variability, coefficient of variation of the method to determine IPA, and an assumed incidence of 20–30% non-responders in the population (data on file, Eli Lilly and Company). Non-responders were also characterized using the definition derived by Gurbel *et al.*[6,12] This approach defines non-responders as those having an absolute difference between baseline MPA and post-treatment MPA (ΔMPA) of <10% with either 5 or 20 μM ADP as the agonist. Non-responder rates among treatment groups were compared using Fisher's exact test.

Results

Patients

A total of 101 subjects were enrolled in the study (Sweden, $n = 83$; USA, $n = 18$). *Figure 1* illustrates the disposition of patients in the study. There were two discontinuations, one due to administration of an incorrect LD (50 mg prasugrel instead of 60 mg) and one at the request of the investigator because of inadequate venous access. Thus, a total of 99 subjects completed the study. All subjects were Caucasian and had CAD. Baseline characteristics and mean baseline MPA responses were consistent across treatment groups (*Table 1*).

Inhibition of platelet aggregation

Figure 2A and *B* illustrates the mean IPA for the LDs and MDs of prasugrel or clopidogrel at all study timepoints by treatment group. At 4 h after the LD on day 1, both the 40 and 60 mg LDs of prasugrel demonstrated at least a doubling of mean

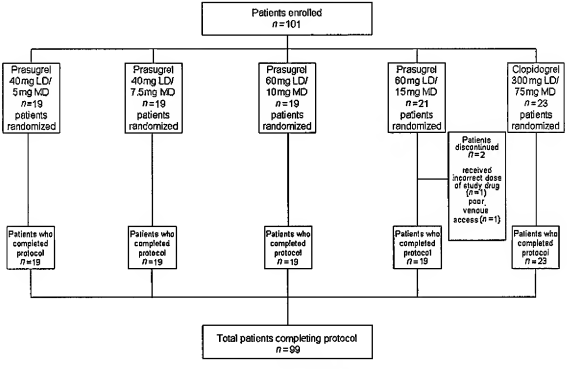

Figure 1 Patient flow through the study.

Table 1 Baseline characteristics of all enrolled subjects

	Prasugrel				Clopidogrel	All subjects ($n = 101$)
LD/MD	40 mg/5 mg ($n = 19$)	40 mg/7.5 mg ($n = 19$)	60 mg/10 mg ($n = 19$)	60 mg/15 mg ($n = 21$)	300 mg/75 mg ($n = 23$)	
Gender						
Male	16	11	18	14	21	80
Female	3	8	1	7	2	21
Age (years, mean ± SD)	65 ± 8.7	65 ± 7.9	65 ± 6.4	63 ± 7.5	61 ± 8.0	64 ± 7.7
Body weight (kg, mean ± SD)	84.7 ± 13.6	84.2 ± 10.0	86.6 ± 14.0	84.7 ± 16.7	86.1 ± 13.1	85.3 ± 13.4
Baseline MPA response with 5 μM ADP (%, mean ± SD)	60.6 ± 16.6	64.3 ± 9.7	65.6 ± 8.7	63.3 ± 10.1	61.4 ± 13.5	63.0 ± 12.0
Baseline MPA response with 20 μM ADP (%, mean ± SD)	72.5 ± 14.1	78.5 ± 9.3	78.2 ± 8.8	74.5 ± 7.5	75.2 ± 7.3	75.7 ± 9.6
Hypertension	10	9	11	10	8	48
Diabetes	2	0	2	4	2	10
Statin	12	12	13	16	16	69
Previous MI	9	10	14	11	12	56
Smokers	3	4	2	3	5	17

LD, loading dose; MD maintenance dose; MPA, maximum platelet aggregation; ACE, angiotensin-converting enzyme; MI, myocardial infarction.

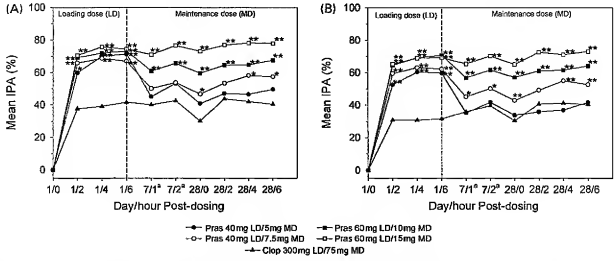

Figure 2 Mean inhibition of aggregation (IPA) induced by ADP over time in each dosing group. Panel A, the agonist is 5 μM ADP. Panel B, the agonist is 20 μM ADP. Values on the left side of the dashed line represent samples obtained pre-loading dose up to 6 h post-LD. Values on the right side of the dashed line represent samples obtained during the MD period. IPA values are adjusted for intersite variability. Statistically significant IPA of prasugrel dose vs. clopidogrel dose at each timepoint is indicated, $^{*}P < 0.05$, $^{**}P < 0.01$. [a]Samples designated as #1 and #2 (see Methods) at the 7 day timepoint. Pras, prasugrel; Clop, clopidogrel.

Table 2 Summary of the inhibition of aggregation (5 and 20 μM ADP agonist) after prasugrel or clopidogrel LD and MD

Hours post-dose	Dose (mg)	ADP 5 μM Mean % IPA (95% CI)	ADP 20 μM Mean % IPA (95% CI)
	LD		
2 h*	Prasugrel/40, $n = 36$	61.7 (55.1, 68.3)**	55.1 (49.1, 61.1)**
	Prasugrel/60, $n = 39$	70.0 (63.7, 76.3)**	64.4 (58.0, 70.8)**
	Clopidogrel/300, $n = 23$	35.9 (28.8, 42.9)	30.2 (22.9, 37.5)
4 h*	Prasugrel/40, $n = 37$	67.8 (62.0, 73.6)**	60.6 (55.1, 66.0)**
	Prasugrel/60, $n = 38$	73.8 (68.3, 79.2)**	68.4 (62.8, 73.9)**
	Clopidogrel/300, $n = 23$	37.0 (24.7, 49.4)	30.0 (22.7, 37.4)
6 h*	Prasugrel/40, $n = 37$	68.6 (63.0, 74.2)**	60.0 (54.4, 65.7)**
	Prasugrel/60, $n = 38$	74.8 (69.3, 80.3)**	69.6 (64.2, 75.0)**
	Clopidogrel/300, $n = 23$	40.7 (30.7, 50.7)	31.1 (23.5, 38.7)
	MD		
Day 7 (sample 2)	Prasugrel/5, $n = 18$	55.9 (42.8, 69.1)	42.9 (33.6, 52.3)
	Prasugrel/7.5, $n = 19$	56.0 (44.6, 67.4)	50.8 (43.5, 58.1)
	Prasugrel/10, $n = 19$	67.5 (57.4, 77.6)[††]	62.2 (55.6, 68.7)[††]
	Prasugrel/15, $n = 19$	78.9 (68.3, 89.6)[††]	71.0 (63.8, 78.2)[††]
	Clopidogrel/75, $n = 23$	45.0 (32.8, 57.2)	40.4 (33.7, 47.1)
Day 28 (0 h)	Prasugrel/5, $n = 19$	41.2 (30.1, 52.2)	34.5 (27.1, 41.9)
	Prasugrel/7.5, $n = 19$	46.6 (36.0, 57.1)[†]	43.4 (36.1, 50.7)[†]
	Prasugrel/10, $n = 19$	59.3 (49.1, 69.5)[††]	57.5 (50.2, 64.8)[††]
	Prasugrel/15, $n = 19$	73.1 (62.8, 83.4)[††]	65.8 (58.7, 72.8)[††]
	Clopidogrel/75, $n = 22$	30.5 (20.4, 40.6)	31.2 (23.9, 38.4)

LD, loading dose; MD, maintenance dose; IPA, inhibition of platelet aggregation.
*$P < 0.01$ for overall test, for both ADP 5 and 20 μM.
**$P < 0.01$ vs. clopidogrel 300 mg LD.
†$p < 0.05$.
††$P < 0.01$ vs. clopidogrel 75 mg MD.

IPA compared with the 300 mg LD of clopidogrel (60.6% and 68.4 vs. 30.0%, respectively; 20 μM ADP, all $P < 0.0001$, *Figure 2B* and *Table 2*). With either 5 or 20 μM ADP, the mean IPA levels for both LDs of prasugrel at 2, 4, and 6 h post-LD were statistically greater than that achieved with the 300 mg LD of clopidogrel (*Table 2*). Although the overall levels of IPA were higher at one site, the relative treatment effects observed were the same at each site (*Figure 3A* and *B*).

During the MD phase, the level of platelet inhibition maintained was dose-related for the four prasugrel doses (*Figures 2A, B,* and *3B*). The prasugrel 10 and 15 mg daily MDs resulted in significantly higher mean levels of IPA than the

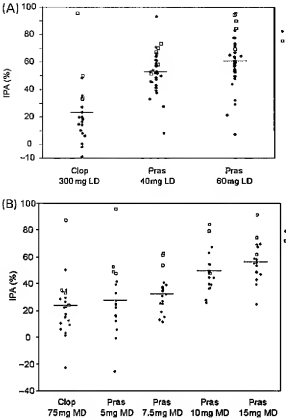

Figure 3 Distribution of IPA with 20 μM ADP as agonist. Panel A illustrates IPA values on day 1 at 4 h post-LD. Panel B illustrates IPA values on day 28 at pre-MD. Stars represent IPA values obtained at site 1 and the open squares represent IPA values obtained at site 2. The horizontal line represents the mean of the entire treatment group. Pras, prasugrel; Clop, clopidogrel.

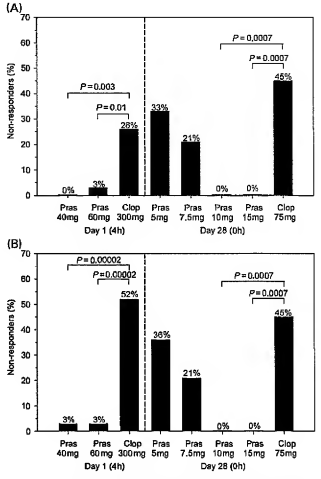

Figure 4 Percentage of non-responders on day 1 at 4 h post-LD, and on day 28 at pre-MD. For this study, a non-responder was defined as a subject with IPA <25% in response to 5 μM ADP (panel A) or <20% in response to 20 μM ADP (panel B). Bars to the left of the dashed line represent the percentage of non-responders 4 h post-LD. Bars to the right of the dashed line represent the percentage of non-responders on day 28 at pre-maintenance dose. Only statistically significant differences (P-value < 0.05) between groups are indicated. Pras, prasugrel; Clop, clopidogrel.

clopidogrel 75 mg MD on both day 7–14 and on day 28 using either 5 or 20 μM ADP ($P \leq 0.01$ at all timepoints, *Table 2*). At pre-dose on day 28, the primary MD timepoint of interest, both the 10 and 15 mg MDs of prasugrel maintained greater mean IPA compared with the 75 mg MD of clopidogrel (57.5% and 65.8 vs. 31.2%, respectively; 20 μM ADP, $P \leq 0.01$, *Figure 2B* and *Table 2*).

Pharmacodynamic non-responders

The percentage of non-responders at 4 h post-LD on day 1 and pre-MD on day 28, as defined by the model-based criteria of IPA <25% in response to 5 μM ADP or IPA <20% in response to 20 μM ADP is illustrated in *Figure 4*.

Adverse events

The majority of adverse events were rated as mild in severity and no subject discontinued study drug dosing due to an adverse event. Only one patient (receiving prasugrel 5 mg MD + aspirin) was classified as having a serious adverse event after being hospitalized on day 29 because of unstable angina.

The number of bruising and minor bleeding events were similar in the three lower prasugrel dose groups and the clopidogrel group (*Table 3*). In the highest prasugrel MD group (15 mg), the increase in minor bruising (mainly bruises on the extremities at sites of venipuncture or bleeding times) and minor bleeding events (predominantly self-limiting episodes of epistaxis) observed was not statistically significant. No bleeding events required medical intervention or were associated with a decrease in haematocrit. In an exploratory analysis, there was no apparent correlation between the level of IPA achieved and the occurrence of these minor bleeding events.

Discussion

The present trial is the first to examine the dose-dependent pharmacodynamic effects of prasugrel, a new $P2Y_{12}$ ADP receptor antagonist, in an aspirin-treated population with stable atherosclerotic disease. Both prasugrel LDs (40 and 60 mg) achieved significantly higher IPA compared with clopidogrel 300 mg LD. During daily dosing, prasugrel demonstrated dose-dependent IPA, with prasugrel 10 and 15 mg MDs maintaining significantly higher IPA compared with clopidogrel 75 mg MD. In addition, the percentage of

Table 3 Adverse events in all enrolled subjects

	Number of adverse events (number of subjects) [percent of subjects]				
	Prasugrel				Clopidogrel
LD/MD	(40/5 mg) ($n = 19$)	(40/7.5 mg) ($n = 19$)	(60/10 mg) ($n = 19$)	(60/15 mg) ($n = 21$)	(300/75 mg) ($n = 23$)
Bruising [%]	35 (12) [63]	49 (13) [68][a]	34 (12) [63]	47 (15) [71]	25 (11) [48]
Bleeding [%]	2 (2) [11]	5 (4) [21]	3 (2) [11]	12 (6) [29]	7 (5) [22]
Bruising and bleeding [%]	37 (13) [68]	54 (15) [79][a]	37 (13) [68]	59 (17) [81]	31 (15) [65]
Epistaxis [%]	1 (1) [5]	2 (1) [5]	2 (1) [5]	8 (5) [24]	4 (2) [9]

Values in parentheses are the number of patients with the specified adverse event. Values in brackets are the percentage of patients within a treatment group with the specified adverse event. The following events are incorporated under the description of bleeding events: epistaxis, gingival bleeding, haemoptysis, tongue haemorrhage, blister, wound, conjunctival haemorrhage, blood in stool, and haematuria (microscopic). No bleeding events were associated with a decrease in haematocrit. Bruising was most often associated with the study procedures (venipuncture, bleeding times). LD, loading dose; MD, maintenance dose.

[a] The number of events in this treatment group was skewed because of a disproportionately high number of bruises reported by one subject (19 separate adverse events of contusion).

non-responders was significantly lower in patients treated with a prasugrel 40 or 60 mg LD compared with clopidogrel 300 mg (3 vs. 52%, respectively) and a prasugrel 10 or 15 mg MD compared with clopidogrel 75 mg (0 vs. 45%, respectively).

Both drugs were well tolerated with a similar incidence of bruising and bleeding events in the three lower dose prasugrel groups and the clopidogrel group. Minor bruising episodes were common and were frequently associated with the study procedures such as venipuncture. There was a modest increase in the incidence of minor bleeding events in the highest dose prasugrel group. The majority of the bleeding events were considered mild to moderate in severity and did not result in discontinuation of study drug. In this study, there was no observed association between the level of IPA on study drug and incidence of bleeding.

In previously published studies, with 20 μM ADP as the agonist, mean IPA observed with clopidogrel 300 mg LD ranges from ~20 to 40%.[13,14] In this study, with either 5 or 20 μM ADP as the agonist, prasugrel 40 and 60 mg LD achieved at least a doubling of mean IPA compared with a mean IPA of about 30% observed with clopidogrel 300 mg LD.

In recent studies of 600 mg clopidogrel, utilizing 20 μM ADP as the agonist, as we employed in the current study, IPA levels of ~31–32% were reported.[15,16] These IPA values are all substantially lower than the 64% IPA that we report here with the prasugrel 60 mg LD. However, given the lack of standardization in the measurement of IPA, determination of the relative levels of IPA achieved by the 60 mg prasugrel LD and the higher 600 mg clopidogrel LD requires a randomized comparison in a clinical trial (such studies are currently ongoing).

A potentially important observation made in the current study is the apparent lower non-responder rate associated with prasugrel. Although previous studies have used empiric definitions of non-responders,[6,7,9,17] there is to date no consensus on how to define pharmacodynamic non-responders to thienopyridine treatment. In the present study, a non-responder was defined, using a model-based approach, as an individual not achieving ≥20% IPA to 20 μM ADP by 4 h after an LD or at pre-dose timepoints under MD administration. The difference in non-responder definition used in this study is a major reason for the higher percentage of non-responders with clopidogrel 300 mg LD (52%) seen in this study compared with previous studies (25–30%).[6,7]

Using the ΔMPA criteria for non-responders reported by Gurbel *et al.*[6,12] the percentage of clopidogrel non-responders in this study is lower and comparable to the literature (~20% non-responders with the clopidogrel 300 mg LD and 30% with the clopidogrel 75 mg MD), reflecting the lower threshold of platelet inhibition required to be considered a pharmacodynamic responder to clopidogrel with this criteria. Similar to the results obtained using the model-based approach in the current study, the percentage of non-responders for prasugrel using Gurbel's definition was still only 3% in the prasugrel 40 and 60 mg LD groups (and 0, 0, 10, and 20% at the prasugrel MDs of 15, 10, 7.5, and 5.0 mg, respectively).

In addition, in contrast to the results reported by Gurbel *et al.*[6] suggesting a decrease in clopidogrel non-responders over time (from 31% at 5 days to 15% at 30 days), in the present study, there was a persistent high level of non-responders (45%, *Figure 3*) to clopidogrel MD even after 28 days of daily treatment. Although assays for the active metabolites of prasugrel and clopidogrel were not available at the time of the current study, subsequent studies indicate differences in the pharmacokinetic profile of prasugrel are consistent with its greater and more consistent pharmacodynamic response.[18,19]

Some studies have suggested that patients with clopidogrel resistance have an increased risk of subsequent stent thrombosis or other cardiovascular events.[7,9,20] There are several potential mechanisms behind the high percentage of clopidogrel non-responders including variations in the absorption of the prodrug and generation and clearance of the active metabolite.[21] Additional mechanisms for thienopyridine resistance may include differences in receptor expression, differences in post-receptor signalling pathways, and $P2Y_{12}$ receptor polymorphisms that have been demonstrated to contribute to varying degrees of platelet aggregation to ADP.[22]

Study limitations

There were several limitations to this study. At present, there is no agreed upon standard for defining non-responders to platelet inhibition with thienopyridines, thus our model-based methodology must be taken in context with varying approaches to defining non-responders in the literature. In addition, in this short-term study of a small population of stable CAD patients, there was only one clinical endpoint of note (serious adverse event of hospitalization for unstable angina), which makes it difficult to gauge the clinical significance of findings regarding higher levels of IPA and lower non-responder rates with prasugrel.

There was variation in the aggregation responses between the two sites that participated in the study, possibly due to methodological differences or differences in ethnic origins of the patient population leading to potential CYP polymorphisms. However, separate analyses of data from each site still support the higher levels of IPA observed with prasugrel 60 mg LD and 10 mg MD over the clopidogrel 300 mg LD and 75 mg MD, results consistent with subsequent studies and with those reported by other investigators.[6,7,12]

Furthermore, clopidogrel was dosed in an open-label manner; this approach should not have altered IPA responses to clopidogrel, but potentially could have impacted the reporting of adverse events. Finally, we cannot rule out the possibility of different IPA response or non-responder rates with either prasugrel or clopidogrel in an acute treatment situation in contrast to the elective setting in this study.

Conclusion

In conclusion, when added to aspirin in patients with stable atherosclerotic disease, prasugrel achieves significantly greater IPA with a significantly lower percentage of pharmacodynamic non-responders compared with clopidogrel. Prasugrel and clopidogrel were well-tolerated and the adverse event profiles were comparable. This study also helped to characterize the IPA associated with LDs and MDs of prasugrel evaluated in the recently completed JUMBO TIMI-26 phase 2 trial performed in the setting of urgent and elective PCI.[11] These combined findings support the selection of the prasugrel 60 mg LD with a 10 mg MD, currently being evaluated against clopidogrel in the TRIal to Assess Improvement in Therapeutic Outcome by Optimizing Platelet InhibitioN with Prasugrel (TRITON) TIMI-38 phase 3 clinical trial in ACS patients undergoing PCI.

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Conflict of interest: none declared.

References

1. Sharis PJ, Cannon CP, Loscalzo J. The antiplatelet effects of ticlopidine and clopidogrel. *Ann Intern Med* 1998;129:394–405.
2. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK, Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001; 345:494–502.
3. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, Topol EJ, Clopidogrel for the Reduction of Events During Observation (CREDO) Investigators. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA* 2002;288:2411–2420.
4. Popma JJ, Berger P, Ohman EM, Harrington RA, Grines C, Weitz JI. Antithrombotic therapy during percutaneous coronary intervention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004;126:576S–599S.
5. Cutlip DE, Baim DS, Ho KK, Popma JJ, Lansky AJ, Cohen DJ, Carrozza JP Jr, Chauhan MS, Rodriguez O, Kuntz RE. Stent thrombosis in the modern era: a pooled analysis of multicenter coronary stent clinical trials. *Circulation* 2001;103:1967–1971.
6. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;107:2908–2913.
7. Muller I, Besta F, Schulz C, Massberg S, Schomig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003;89:783–787.
8. Neubauer H, Gunesdogan B, Hanefeld C, Spiecker M, Mugge A. Lipophilic statins interfere with the inhibitory effects of clopidogrel on platelet function—a flow cytometry study. *Eur Heart J* 2003; 24:1744–1749.
9. Matetzky S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D, Hod H. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004; 109:3171–3175.
10. Sugidachi A, Asai F, Ogawa T, Inoue T, Kofke H. The *in vivo* pharmacological profile of CS-747, a novel antiplatelet agent with platelet ADP receptor antagonist properties. *Br J Pharmacol* 2000; 129:1439–1446.
11. Wiviott SD, Antman EM, Winters KJ, Weerakkody G, Behounek BD, Carney RJ, Lazzam C, McCabe CH, Braunwald E. A randomized comparison of prasugrel (CS-747, LY640315), a novel thienopyridine $P2Y_{12}$ antagonist, to clopidogrel in percutaneous coronary intervention: results of the Joint Utilization of Medications to Block Platelets Optimally (JUMBO)-TIMI 26 Trial. *Circulation* 2005; 111:3366–3373.
12. Gurbel PA, Bliden KP. Durability of platelet inhibition by clopidogrel. *Am J Cardiol* 2003;91:1123–1125.
13. Muller I, Seyfarth M, Rudiger S, Wolf B, Pogatsa-Murray G, Schomig A, Gawaz M. Effect of a high loading dose of clopidogrel on platelet function in patients undergoing coronary stent placement. *Heart* 2001;85:92–93.
14. Ernst NM, Suryapranata H, Miedema K, Slingerland RJ, Ottervanger JP, Hoorntje JC, Gosselink AT, Dambrink JH, de Boer MJ, Zijlstra F, van't Hof AW. Achieved platelet aggregation inhibition after different antiplatelet regimens during percutaneous coronary intervention for ST-segment elevation myocardial infarction. *J Am Coll Cardiol* 2004;44:1187–1193.
15. Kastrati A, vonBeckerath N, Joost A, Pogatsa-Murry G, Gorchakova O, Schomig A. Loading with 600 mg clopidogrel in patients with coronary artery disease with and without chronic clopidogrel therapy. *Circulation* 2004;110:1–4.
16. Hochholzer W, Trenk D, Frundi D, Blanke P, Fischer B, Andris K, Bestehorn HP, Buttner HJ, Neumann FJ. Time dependence of platelet inhibition after a 600 mg loading dose of clopidogrel in a large, unselected cohort of candidates for percutaneous coronary intervention. *Circulation* 2005;111:2560–2564.
17. Samara WM, Bliden KP, Tantry US, Gurbel PA. The difference between clopidogrel responsiveness and post-treatment platelet reactivity. *Thromb Res* 2005;115:89–94.
18. Payne CD, Brandt JT, Weerakkody GJ, Farid NA, Small DS, Ernest CS *et al.* Superior inhibition of platelet aggregation following a loading dose of CS-747 (Prasugrel, LY640315) vs. clopidogrel: correlation with the

pharmacokinetics of active metabolite generation (abstract no.: P0952). *J Thromb Haemost* 2005;3(Suppl. 1).
19. Sugidachi A, Ogawa T, Hasegawa M, Nakanishi Y, Suzuki N, Kurihara A *et al.* Active metabolites of CS-747 (prasugrel, LY640315) and clopidogrel show similar *in vitro* P2Y12 receptor blockade but greater *in vivo* potency of CS-747 (abstract no.: P1109). *J Thromb Haemost* 2005; 3(Suppl. 1).
20. Barragan P, Bouvier JL, Roquebert PO, Macaluso G, Commeau P, Comet B, Lafont A, Camoin L, Walter U, Eigenthaler M. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. *Catheter Cardiovas Interv* 2003;59:295–302.
21. Bates ER, Lau WC. Controversies in antiplatelet therapy for patients with cardiovascular disease. *Circulation* 2005;111:e267–e2271.
22. Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, Gaussem P. Adenosine diphosphate-induced platelet aggregation is associated with *P2Y12* gene sequence variations in healthy subjects. *Circulation* 2003;108:989–995.

Exhibit I

Genetic variants in *ABCB1* and *CYP2C19* and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON–TIMI 38 trial: a pharmacogenetic analysis

Jessica L Mega, Sandra L Close*, Stephen D Wiviott, Lei Shen, Joseph R Walker, Tabassome Simon, Elliott M Antman, Eugene Braunwald, Marc S Sabatine*

Summary

Background Clopidogrel and prasugrel are subject to efflux via P-glycoprotein (encoded by *ABCB1*, also known as *MDR1*). *ABCB1* polymorphisms, particularly 3435C→T, may affect drug transport and efficacy. We aimed to assess the effect of this polymorphism by itself and alongside variants in *CYP2C19* on cardiovascular outcomes in patients treated with clopidogrel or prasugrel in TRITON–TIMI 38. We also assessed the effect of genotype on the pharmacodynamic and pharmacokinetic properties of these drugs in healthy individuals.

Methods We genotyped *ABCB1* in 2932 patients with acute coronary syndromes undergoing percutaneous intervention who were treated with clopidogrel (n=1471) or prasugrel (n=1461) in the TRITON–TIMI 38 trial. We evaluated the association between *ABCB1* 3435C→T and rates of the primary efficacy endpoint (cardiovascular death, myocardial infarction, or stroke) until 15 months. We then assessed the combined effect of *ABCB1* 3435C→T genotype and reduced-function alleles of *CYP2C19*. 321 healthy individuals were also genotyped, and we tested the association of genetic variants with reduction in maximum platelet aggregation and plasma concentrations of active drug metabolites.

Findings In patients treated with clopidogrel, *ABCB1* 3435C→T genotype was significantly associated with the risk of cardiovascular death, myocardial infarction, or stroke (p=0·0064). TT homozygotes had a 72% increased risk of the primary endpoint compared with CT/CC individuals (Kaplan-Meier event rates 12·9% [52 of 414] vs 7·8% [80 of 1057 participants]; HR 1·72, 95% CI 1·22–2·44, p=0·002). *ABCB1* 3435C→T and *CYP2C19* genotypes were significant, independent predictors of the primary endpoint, and 681 (47%) of the 1454 genotyped patients taking clopidogrel who were either *CYP2C19* reduced-function allele carriers, *ABCB1* 3435 TT homozygotes, or both were at increased risk of the primary endpoint (HR 1·97, 95% CI 1·38–2·82, p=0·0002). In healthy participants, 3435 TT homozygotes had an absolute reduction in maximum platelet aggregation with clopidogrel that was 7·3 percentage points less than for CT/CC individuals (p=0·0127). *ABCB1* genotypes were not significantly associated with clinical or pharmacological outcomes in patients with an acute coronary syndrome or healthy individuals treated with prasugrel, respectively.

Interpretation Individuals with the *ABCB1* 3435 TT genotype have reduced platelet inhibition and are at increased risk of recurrent ischaemic events during clopidogrel treatment. In patients with acute coronary syndromes who have undergone percutaneous intervention, when both *ABCB1* and *CYP2C19* are taken into account, nearly half of the population carries a genotype associated with increased risk of major adverse cardiovascular events while on standard doses of clopidogrel.

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Introduction

In patients presenting with acute coronary syndromes and in those undergoing percutaneous coronary interventions with stenting, dual antiplatelet treatment with aspirin and the thienopyridine clopidogrel is the guideline-approved standard of care.[1,2] As such, clopidogrel is one of the most frequently prescribed drugs worldwide. However, the pharmacodynamic response to clopidogrel varies substantially between patients,[3] and individuals with low platelet inhibition during treatment with clopidogrel are at increased risk of cardiovascular events.[4] Prasugrel is a third-generation thienopyridine that achieves greater platelet inhibition with less variability between patients than does clopidogrel.[5] In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction (TRITON–TIMI) 38, treatment with prasugrel compared with clopidogrel resulted in a significantly lower rate of ischaemic events and more bleeding.[6]

Both clopidogrel and prasugrel are prodrugs that need intestinal absorption and subsequent biotransformation to active metabolites by cytochrome P450 enzymes. In several studies, reduced-function genetic variants in *CYP2C19* (located on chromosome 10) have been associated with reduced concentrations of active drug metabolite, diminished platelet inhibition, and higher rates of adverse cardiovascular events in the setting of treatment with clopidogrel, but not prasugrel.[7–15] To that end, the US Food and Drug Administration has

incorporated *CYP2C19* genetic information into the updated clopidogrel label in the form of a boxed warning noting that carriers of two reduced-function *CYP2C19* alleles have a diminished response to standard doses of clopidogrel.

Additionally, a key protein involved in thienopyridine absorption is the efflux pump P-glycoprotein, which is encoded by *ABCB1* (also known as *MDR1*, located on chromosome 7). P-glycoprotein is an ATP-dependent efflux pump that transports various molecules across extracellular and intracellular membranes. It is expressed, among other places, on intestinal epithelial cells, where increased expression or function can affect bioavailability of drugs that are substrates. Previous research suggests that when treated with clopidogrel, individuals with genetic variants in *ABCB1* (specifically those who are TT homozygotes for the 3435C→T variant) have reduced concentrations of the active drug metabolite[16] and increased rates of adverse clinical outcomes.[20] Further investigation into the effect of this polymorphism on outcomes in patients treated with clopidogrel, the effect in relation to *CYP2C19* reduced-function variants, and the effect in those treated with the third-generation thienopyridine prasugrel is needed.

We genotyped a subset of patients in the TRITON–TIMI 38 trial who provided samples for genetic analysis with the aim of assessing the association between the *ABCB1* 3435C→T polymorphism and adverse cardiovascular outcomes during treatment with clopidogrel or prasugrel. To obtain supporting pharmacological data, *ABCB1* genotyping was also done in healthy individuals in whom platelet inhibition and drug concentrations were measured in response to clopidogrel or prasugrel. We also assessed the contribution of the *ABCB1* 3435C→T polymorphism in the context of *CYP2C19* status to elucidate the independent contribution of variants in these two genes.

Methods

Patients

The design and primary results of the TRITON–TIMI 38 trial have been described previously.[4] Patients with acute coronary syndromes undergoing planned percutaneous coronary interventions were randomly allocated to treatment with clopidogrel (300 mg loading dose followed by 75 mg daily) or prasugrel (60 mg loading dose followed by 10 mg daily) for up to 15 months. We undertook this pharmacogenetic analysis in a TRITON–TIMI 38 genetic substudy that included 2932 patients who both provided a genetic sample and had *ABCB1* genotyped (n=1471 for clopidogrel and n=1461 for prasugrel). This study was approved by institutional review boards, and written informed consent was obtained from all participants.

Healthy participants in seven studies (n=321) involving treatment with clopidogrel or prasugrel, or both, were included in the pharmacodynamic and pharmacokinetic analyses (webappendix pp 1 and 4).[5] These studies were approved by institutional review boards, and written informed consent was obtained from all participants.

See Online for webappendix

Procedures

In the TRITON–TIMI 38 study, the prespecified primary efficacy endpoint was a composite of cardiovascular death, myocardial infarction, or stroke.[4] A secondary endpoint was definite or probable stent thrombosis as defined by the Academic Research Consortium.[17] Safety endpoints included TIMI major or minor bleeding not related to coronary artery bypass grafting. These outcomes were adjudicated by a clinical events committee unaware of treatment assignment.

Among the healthy participants, pharmacodynamic response was assessed by use of light transmission aggregometry in response to 20 μmol/L ADP, and was expressed as absolute reduction in maximum platelet aggregation from baseline to 4 h. Plasma concentrations of clopidogrel and prasugrel active drug metabolite were measured by liquid chromatography with mass spectrometry.[18] The area under the plasma concentration–time curve was analysed by the log-linear trapezoidal method from time of dose to the 4-h measurable concentration (AUC_{0-4}).

Genotyping for *ABCB1* was completed with the Affymetrix Targeted Human DMET 1.0 Assay (Affymetrix, Santa Clara, CA, USA) and Illumina Infinium Beadchip Assay (Illumina, San Diego, CA, USA) to minimise missing data.[19] On the basis of previous studies,[20,21] the main variant of interest was 3435C→T (rs1045642), and participants were classified as homozygous for the C allele (CC), heterozygous (CT), or homozygous for the T allele (TT). Since some in-vitro studies have also assessed a haplotype consisting of 3435C→T and two other *ABCB1* variants, 2677G→T/A (rs2032582) and 1236C→T (rs1128503), we also genotyped these polymorphisms (webappendix p 1).[22] Genotypes were in Hardy-Weinberg equilibrium (webappendix p 5).

Because genetic variation in *CYP2C19* has been associated with pharmacological response and cardiovascular outcomes in patients taking clopidogrel,[8–11,23–25] we assessed the combined effect of genetic variants in *CYP2C19* and *ABCB1* 3435C→T. For *CYP2C19*, participants were genotyped and divided into two groups on the basis of whether they had at least one reduced-function allele (termed carriers) or no reduced-function alleles (termed non-carriers).[8]

Statistical analysis

Analyses were done with SAS (version 9.1) and S-PLUS (version 8.0). On the basis of previous studies, the primary objective was to investigate the association between *ABCB1* 3435C→T genotypes and rates of the primary efficacy endpoint in patients in the TRITON–TIMI 38 study. For consistency with the main trial analyses, the Gehan-

Wilcoxon test was used for the primary efficacy endpoint and log-rank for other endpoints. Event rates were expressed as Kaplan-Meier estimates at 15 months.[25] Hazard ratios and 95% CIs were calculated on the basis of Cox proportional hazards regression models with clinical syndrome (non-ST-elevation *vs* ST-elevation acute coronary syndromes) as a stratification factor. Two-sided p values were calculated to test for differences in cardiovascular event rates between patients stratified by genotype. If a significant association for the primary efficacy evaluation was identified in patients treated with clopidogrel, additional efficacy endpoints were also tested, including the hazards for the components of the composite primary endpoint, the primary endpoint at 30 days, and stent thrombosis. In terms of safety endpoints, TIMI major or minor bleeding not related to coronary artery bypass grafting was assessed until 15 months. Parallel analyses were done for patients allocated treatment with prasugrel.

To elucidate further the contribution of *ABCB1* variants, the associations between additional *ABCB1* genotypes (2677G→T/A, 1236C→T, and the haplotype that included 1236C→T, 2677G→T/A, and 3435C→T; webappendix p 1) and cardiovascular outcomes were tested in each treatment group, with the same methods. We then evaluated 3435C→T in the context of *CYP2C19*. We created Cox proportional hazards regression models examining 3435C→T that were adjusted for *CYP2C19* reduced-function allele status as well as models that stratified patients into four groups on the basis of 3435C→T genotype and *CYP2C19* reduced-function allele status. We did a meta-analysis that included results from FAST-MI[10] by combining HRs for each study using a fixed-effects model with weighting based on inverse variance.

We tested the associations between genetic variation and pharmacodynamic and pharmacokinetic parameters using likelihood ratio tests based on linear regression or mixed-effects models. The primary outcomes were platelet inhibition (change in maximum platelet aggregation) and exposure to active drug metabolite [$\log(AUC_{0-t})$]. The models contained subject as a random effect when repeated measures were present, genotype as the predictor of main interest, and other fixed effects including study, dose, and ethnic origin, and for pharmacodynamics, maximum platelet aggregation at baseline. Other demographic variables, including bodyweight, age, sex, and smoking, were included as judged to be appropriate for each drug, as has been done previously.[4] Additional models were also created with adjustment for *CYP2C19*.

Role of the funding source

The TRITON–TIMI 38 genetic study was designed and undertaken in collaboration between the TIMI Study Group and the sponsors. The academic authors directed and had access to all the analyses and the full clinical database, wrote all drafts of the report, decided to publish the results, and vouch for the accuracy and completeness of the data.

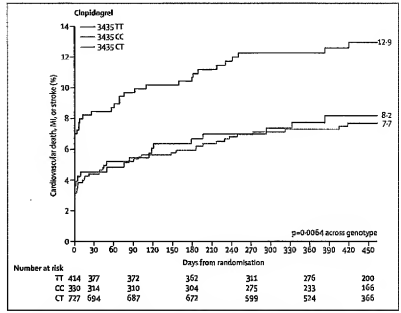

Figure 1: **ABCB1 3435C→T and cardiovascular outcomes in patients treated with clopidogrel**
Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for each genotype, with a p value across genotype.

Results

For the 2932 patients in the TRITON–TIMI 38 genetic substudy, the average age was 60·2 (SD 10·9) years, 831 (28%) were women, 2064 (70%) presented with non-ST-elevation acute coronary syndromes, and 868 (30%) presented with ST-elevation myocardial infarction. For *ABCB1* 3435C→T, 804 (27%) participants in the genetic study population were TT homozygotes, 1459 (50%) CT heterozygotes, and 669 (23%) CC homozygotes. Baseline characteristics in the TRITON–TIMI 38 trial by 3435C→T genotype are shown in the webappendix p 6.

In patients in the TRITON–TIMI 38 genetic substudy who were allocated to treatment with clopidogrel (n=1471), 3435C→T genotype was significantly associated with risk of the primary endpoint of cardiovascular death, myocardial infarction, or stroke (p=0·0064; figure 1). TT homozygotes were at significantly increased risk compared with CC individuals (HR 1·69, 95% CI 1·05–2·72); CT heterozygotes were at similar risk to CC individuals (HR 0·94, 0·58–1·51). Thus, TT homozygotes for 3435C→T had a 72% increased risk of the primary endpoint compared with CT/CC individuals (Kaplan-Meier event rates 12·9% [52 of 414] *vs* 7·8% [80 of 1057 participants]; HR 1·72, 95% CI 1·22–2·44, p=0·002) when assessed until 15 months. Among 3435 TT versus CT/CC patients, the HR for cardiovascular death was 1·63 (Kaplan-Meier event rates 1·3% [five of 414] *vs* 0·9% [eight of 1057]; 95% CI 0·53–4·98, p=0·388), that for non-fatal myocardial infarction was 1·82 (12·0% [48 of 414] *vs* 6·8% [70 of 1057]; 1·26–2·62, p=0·0013), and that

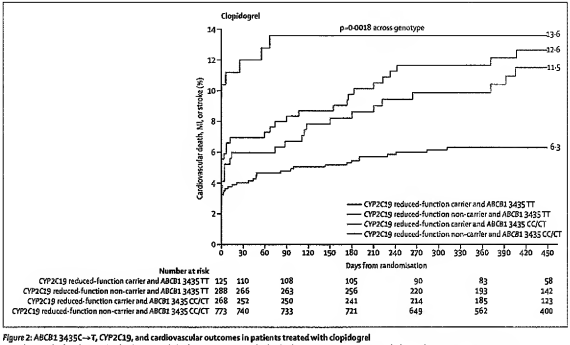

Figure 2: ABCB1 3435C→T, *CYP2C19*, and cardiovascular outcomes in patients treated with clopidogrel
Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for the four genotype categories, with the p value across genotype category.

for non-fatal stroke was 1·66 (0·6% [two of 414] *vs* 0·3% [three of 1057 participants]; 0·28–9·93, p=0·575). Moreover, the increased risk was evident by 30 days, by which time the risk of the primary endpoint for the 3435 TT homozygotes was roughly twice as high as that for CT/CC individuals (Kaplan-Meier event rates 8·5% [35 of 414] *vs* 4·5% [47 of 1057 participants]; HR 1·96, 95% CI 1·26–3·03, p=0·0022).

Rates of stent thrombosis did not differ significantly between 3435 TT and CT/CC individuals (Kaplan-Meier event rates 1·3% [five of 396] *vs* 1·3% [12 of 1004 participants]; HR 1·07, 95% CI 0·38–3·04, p=0·9). Rates of TIMI major or minor bleeding not related to coronary artery bypass grafting did not differ significantly by genotype (Kaplan-Meier event rates 3·6% [15 of 414] TT homozygotes *vs* 2·5% [26 of 1052] CT/CC individuals; HR 1·49, 95% CI 0·79–2·82, p=0·214).

To elucidate further the contribution of *ABCB1* variants in the setting of treatment with clopidogrel, two other *ABCB1* polymorphisms were explored: 2677G→T/A and 1236C→T. Neither variant was significantly associated with risk of cardiovascular death, myocardial infarction, or stroke (webappendix pp 2, 7, and 8). We constructed haplotypes using the three loci (1236C→T, 2677G→T/A, and 3435C→T) and did not identify associations beyond what was seen for 3435C→T alone (webappendix p 2).

In a model containing both *ABCB1* 3435C→T genotype and *CYP2C19* reduced-function allele carrier status in patients in the TRITON–TIMI 38 genetic substudy treated with clopidogrel, both variants were significant, independent predictors of cardiovascular death, myocardial infarction, or stroke (*ABCB1* 3435 TT *vs* CT/CC, HR 2·01, 95% CI 1·30–3·11, p=0·0017; *CYP2C19* reduced-function allele carrier *vs* non-carrier, HR 1·77, 1·11–2·80, p=0·0155). When the participants were divided into four groups on the basis of *ABCB1* 3435C→T genotype and *CYP2C19* status (figure 2), the 773 patients (53% of 1454 genotyped) who did not carry at-risk genotypes in either gene had a low rate of cardiovascular death, myocardial infarction, or stroke at 15 months (Kaplan-Meier event rate 6·3%, 48 of 773 participants). By contrast, event rates were significantly higher in the 681 patients (47% of 1454 genotyped) who were either carriers of a *CYP2C19* reduced-function allele only (Kaplan-Meier event rate 11·5%, 29 of 268 participants), *ABCB1* 3435 TT homozygotes only (Kaplan-Meier event rate 12·6%, 35 of 288 participants), or both (Kaplan-Meier event rate 13·6%, 17 of 125 participants) (pooled HR 1·97, 95% CI 1·38–2·82, p=0·0002).

When we examined the early timepoint of 30 days, individuals who did not carry either at-risk variant were at low risk (Kaplan-Meier event rate 4·0%, 31 of 773 participants), those who were either *ABCB1* 3435 TT homozygotes or carriers of a *CYP2C19* reduced-function allele were at intermediate risk (Kaplan-Meier event rate 7·0% [20 of 288 participants] for TT homozygotes and 6·0% [16 of 268 participants] for carriers of a reduced-function allele; pooled HR 1·64, 95% CI 1·01–2·65, p=0·0441 *vs* carriers of neither), and individuals who were both *CYP2C19* reduced-function allele carriers and *ABCB1* 3435 TT homozygotes were at high risk (Kaplan-Meier event rate 12·0%, 15 of 125 participants; HR 3·16, 95% CI 1·71–5·85, p=0·0003 *vs* carriers of neither).

There was no significant association between *ABCB1* 3435C→T genotype and risk of cardiovascular death, myocardial infarction, or stroke among patients in the TRITON–TIMI 38 genetic substudy who had been allocated to prasugrel (n=1461; figure 3 and webappendix p 2). Specifically, TT homozygotes did not have a significantly higher risk of the primary efficacy endpoint of cardiovascular death, myocardial infarction, or stroke than did CT/CC carriers (Kaplan-Meier event rates 11·0% [41 of 390] *vs* 8·7% [91 of 1071 participants]; HR 1·25, 95% CI 0·86–1·81, p=0·235) when assessed until 15 months. Rates of TIMI major or minor bleeding not related to coronary artery bypass grafting did not differ significantly by *ABCB1* 3435C→T genotype (webappendix p 2). In terms of other *ABCB1* variants, the 2677G→T/A and 1236C→T genotypes overall were not significantly associated with risk of the primary efficacy endpoint in patients treated with prasugrel, although there was a non-significant trend for 2677 TT homozygotes versus CT/CC individuals to be at increased risk (Kaplan-Meier event rates 11·8% [29 of 253] *vs* 8·8% [94 of 1104 participants]; HR 1·38, 95% CI 0·91–2·09, p=0·1290; webappendix pp 3, 9, and 10). When we divided patients on the basis of *ABCB1* 3435C→T genotypes and *CYP2C19* status, rates of cardiovascular death, myocardial infarction, or stroke at 15 months were similar in the four groups (p=0·4851, figure 4).

In healthy participants treated with clopidogrel, *ABCB1* 3435 TT homozygotes had a diminished pharmacodynamic effect, with an absolute reduction in maximum platelet aggregation in response to a clopidogrel loading dose that was 7·3 percentage points lower (ie, less platelet inhibition) than that seen in CT/CC individuals (p=0·0127). After adjustment for *CYP2C19* genotype, the response was 6·6 percentage points lower (p=0·022). The pharmacodynamic effects of clopidogrel on TT carriers were discernible only after a loading dose (for both 300 mg and 600 mg); no significant association was identified during maintenance dosing. There was no significant association between 3435C→T genotype and exposure to clopidogrel active metabolite concentrations. In prasugrel-treated individuals, 3435C→T genotype was not significantly associated with platelet response (1·3 percentage points higher; p=0·4345) or exposure to prasugrel's active metabolite. There was no relation between genotype status for either 2677G→T/A or 1236C→T and pharmacodynamic outcomes in patients treated with clopidogrel or prasugrel.

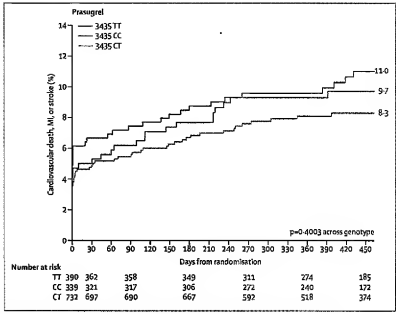

Figure 3: **ABCB1 3435C→T and cardiovascular outcomes in patients treated with prasugrel**
Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for each genotype, with a p value across genotype.

Discussion

The pharmacological and clinical response to clopidogrel varies widely between patients, and genetic variants in *CYP2C19* have been shown to affect the response. P-glycoprotein is important in drug transport, and pharmacogenetic interactions with various classes of drugs have been suggested.[16] Our findings show that TT homozygotes for the 3435C→T variant in *ABCB1* (27% of the study population), as compared with CT/CC individuals, had reduced platelet inhibition with a clopidogrel loading dose in a healthy study population and a significantly increased risk of adverse cardiovascular events during treatment with clopidogrel in patients with acute coronary syndromes undergoing percutaneous coronary intervention. When we considered *ABCB1* 3435C→T genotype in the context of *CYP2C19* reduced-function allele status in patients treated with clopidogrel, we showed that variants in the two genes offered significant, independent information about the risk of cardiovascular death, myocardial infarction, or stroke. Conversely, there were no significant associations between the *ABCB1* variants tested and the response to prasugrel.

ABCB1 encodes the P-glycoprotein efflux transporter. Clopidogrel is a P-glycoprotein substrate, and inhibition of P-glycoprotein affects the bioavailability of clopidogrel.[16] The 3435C→T variant in *ABCB1* is one of the most studied polymorphisms in pharmacogenetic research, and has been associated with altered disposition of several drugs.[16] Although a genome-wide association study identified only *CYP2C19* as being associated with the pharmacodynamic response to clopidogrel, that study showed that platelet response to clopidogrel was highly heritable and was not entirely accounted for by *CYP2C19* status, suggesting that additional genetic variants might be relevant. In a study of patients treated with clopidogrel after elective percutaneous coronary intervention, 3435 TT homozygotes had significantly lower active clopidogrel metabolite concentrations than did CT/CC

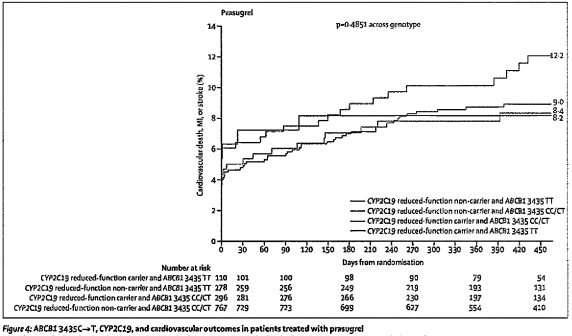

Figure 4: ***ABCB1*** **3435C→T, CYP2C19, and cardiovascular outcomes in patients treated with prasugrel**
Cumulative risk of cardiovascular death, myocardial infarction (MI), or stroke for the four genotype categories, with the p value across genotype category.

individuals, suggesting increased intestinal efflux possibly mediated by higher P-glycoprotein expression associated with the 3435 TT genotype.[16] Although evidence on P-glycoprotein expression and activity is inconsistent, mRNA expression in duodenal enterocytes has been reported to be two-to-three-times higher for the *ABCB1* 3435 TT genotype than for either the CC or CT genotype.[17–19] In the healthy participants in our analysis, TT homozygotes had an absolute reduction in maximum platelet aggregation after a loading dose of clopidogrel that was 7·3 percentage points lower (ie, decreased platelet inhibition) than in CC or CT individuals. Although we did not record a significant association between 3435C→T genotype and pharmacokinetic data, other researchers have shown this relation, and the differences in results could be related to patients, methods, and single-centre versus multicentre study design.

In terms of clinical outcomes, we showed that *ABCB1* 3435 TT homozygotes had a 72% increased risk of adverse cardiovascular events compared with CT/CC individuals in the setting of treatment with clopidogrel in TRITON–TIMI 38. Likewise, in a previous study in patients receiving clopidogrel after an acute myocardial infarction, those who were 3435 TT homozygotes had an increase of about 70% in cardiovascular events during follow-up.[16] In that previous study, however, 3435 CT heterozygotes were also at increased risk of adverse cardiovascular events, albeit less so than were TT homozygotes; the differences in the findings could be attributable to the patient populations. Combination of the results of the previous study and our findings yielded an apparent graded allele-dose response with an HR for adverse cardiovascular events of 1·29 (95% CI 0·99–1·69) for 3435 CT versus CC individuals and a HR of 1·70 (1·28–2·26) for 3435 TT versus CC individuals. Incorporation of clinical data from other studies will be helpful to further refine the risk estimates. In our analysis, 2677G→T/A and 1236C→T genotypes did not add additional significant information. Nonetheless, further basic genetic pharmacology studies could be helpful to further define the actual functional *ABCB1* variants and the most appropriate genetic model with respect to response to clopidogrel.

In our study, assessment of the contribution of *ABCB1* variants in the context of *CYP2C19* showed that variants in the two genes offered complementary information about cardiovascular risk. When we divided patients into four groups on the basis of *ABCB1* 3435C→T and *CYP2C19* reduced-function allele status, rates of cardiovascular death, myocardial infarction, or stroke until 15 months were nearly twice as high in the study population who were either carriers of a *CYP2C19* reduced-function allele, 3435 TT homozygotes, or both, compared with individuals who did not carry either. Moreover, when both *ABCB1* and *CYP2C19* were taken into account, in this population of patients with an acute coronary syndrome undergoing percutaneous coronary intervention, nearly half of the population carried a genotype associated with increased risk of major adverse cardiovascular events during treatment with standard doses of clopidogrel.

In patients taking prasugrel in TRITON–TIMI 38, *ABCB1* 3435C→T polymorphisms were not significantly associated with cardiovascular outcomes. Likewise, in

healthy participants, no associations between the 3435C→T variant and pharmacokinetic and pharmacodynamic outcomes were seen with prasugrel. The rapid metabolism of prasugrel might mitigate the genetic effect of *ABCB1* 3435C→T polymorphisms, even though the drug is subject to the P-glycoprotein system. Among participants treated with prasugrel, there was a non-significant trend towards 2677 TT homozygotes having higher rates of adverse cardiovascular events compared with the rest of the population. No association was seen with 2677G→T/A and the pharmacological data. Future studies will assist in further examination of these exploratory findings.

There are several limitations to this analysis. First, few non-Caucasian individuals were included in these studies, and future investigations in other populations would be useful. Second, because of the sample handling and the need for repeat measurements for the pharmacokinetic and pharmacodynamic assessments, these investigations were done in healthy individuals, not in the acute clinical trial study population. Third, in our clinical outcomes study, patients treated with clopidogrel received a 300 mg loading dose and 75 mg daily maintenance dose, and patients treated with prasugrel received a 60 mg loading dose and 10 mg daily maintenance dose; we cannot comment on the effect of *ABCB1* genetic variants in patients receiving other doses of these drugs. Fourth, the number of bleeding and stent thrombosis events was small, and our analysis had restricted power to detect an association between the tested *ABCB1* variants and these outcomes. Additional studies that include more such events will be particularly important to further elucidate the relations between *ABCB1* genetic variants and outcomes. Finally, there might be other genetic variants affecting the association between treatment with clopidogrel and cardiovascular outcomes.

In conclusion, we found that *ABCB1* 3435 TT homozygotes had an increased risk of adverse cardiovascular outcomes during treatment with clopidogrel after an acute coronary syndrome and percutaneous coronary intervention. Thus, the association between *ABCB1* polymorphisms and ischaemic risk in patients treated with clopidogrel has been noted now in several pharmacological and clinical outcomes studies. Our analysis also shows that the pharmacogenetic effects of *ABCB1* 3435C→T are independent of and complementary to those of *CYP2C19*. As clinicians, professional societies, and patients integrate information about genetic factors affecting the response to thienopyridines, the roles of both *ABCB1* and *CYP2C19* should be considered.

Contributors

JLM and MSS conceived of and designed the research. SDW and EMA acquired the data. JLM, SLC, and MSS analysed and interpreted the data. LS did statistical analyses. JLM and MSS drafted the initial report. JRW and EB participated in funding and supervision. JLM, SLC, SDW, JRW, TS, EMA, EB, and MSS made critical revisions to the report for important intellectual content.

Conflicts of interest

The TIMI Study Group receives research grant support from Daiichi Sankyo, Eli Lilly, Sanofi-Aventis, Bristol-Myers Squibb, AstraZeneca, Schering-Plough/Merck, Johnson & Johnson, and Bayer Healthcare. Additionally, JLM is supported in part by grant K99/R00 HL098461-01 from the National Institutes of Health and reports consulting fees from Sanofi-Aventis, Bristol-Myers Squibb, and AstraZeneca. SDW reports consulting fees from Sanofi-Aventis, Bristol-Myers Squibb, AstraZeneca, ARENA, Medco, and Portola, and lecture fees for CME from Schering-Plough, Daiichi Sankyo, Eli Lilly, Novartis, and AstraZeneca. TS reports research grant support from Servier, Pfizer, Daiichi Sankyo, Eli Lilly, Sanofi-Aventis, AstraZeneca, and Caisse d'Assurance Maladie and consulting fees from Eli Lilly, Daiichi Sankyo, Sanofi-Aventis, Bristol-Myers Squibb, and AstraZeneca. EMA has no additional relationships to disclose. EB reports consulting fees from Daiichi Sankyo and lecture fees from Schering-Plough and Merck. MSS reports consulting fees from AstraZeneca, Bristol-Myers Squibb, Sanofi-Aventis, Daiichi Sankyo, and Eli Lilly and lecture fees from Bristol-Myers Squibb, Sanofi-Aventis, and Eli Lilly. JRW is an employee of Daiichi Sankyo and holds equity ownership or stock options therein. SLC is a former employee of Eli Lilly and holds equity ownership or stock options therein. LS is an employee of Eli Lilly and holds equity ownership or stock options therein.

References

1 Anderson JL, Adams CD, Antman EM, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-Elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction) developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. *J Am Coll Cardiol* 2007; 50: e1–157.

2 Kushner FG, Hand M, Smith SC Jr, et al. 2009 Focused updates: ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction (updating the 2004 Guideline and 2007 Focused Update) and ACC/AHA/SCAI Guidelines on Percutaneous Coronary Intervention (updating the 2005 Guideline and 2007 Focused Update): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2009; 120: 2271–306.

3 Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003; 107: 2908–13.

4 Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006; 48: 1742–50.

5 Wiviott SD, Trenk D, Frelinger AL, et al. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. *Circulation* 2007; 116: 2923–32.

6 Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007; 357: 2001–15.

7 Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G→A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008; 51: 1925–34.

8 Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; 360: 354–62.

9 Collet JP, Hulot JS, Pena A, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009; 373: 309–17.

10 Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009; 360: 363–75.

11 Giusti B, Gori AM, Marcucci R, et al. Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. *Am J Cardiol* 2009; 103: 806–11.

12 Sibbing D, Stegherr J, Latz W, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J* 2009; 30: 916–22.

13 Shuldiner AR, O'Connell JR, Bliden KP, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA* 2009; 302: 849–57.

14 Anderson JL, Mower CP, Horne BD, et al. Carriage of the *CYP2C19*2* allele increases one-year risk of myocardial infarction among recipients of drug-eluting stents treated with clopidogrel. *J Am Coll Cardiol* 2009; 53 (10 suppl A): A27.

15 Mega JL, Close SL, Wiviott SD, et al. Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation* 2009; 119: 2553–60.

16 Taubert D, von Beckerath N, Grimberg G, et al. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther* 2006; 80: 486–501.

17 Wiviott SD, Braunwald E, McCabe CH, et al, for the TRITON–TIMI 38 Investigators. Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITON-TIMI 38 trial: a subanalysis of a randomised trial. *Lancet* 2008; 371: 1353–63.

18 Farid NA, McIntosh M, Garofolo F, et al. Determination of the active and inactive metabolites of prasugrel in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2007; 21: 169–79.

19 Dumaual C, Miao X, Daly TM, et al. Comprehensive assessment of metabolic enzyme and transporter genes using the Affymetrix Targeted Genotyping System. *Pharmacogenomics* 2007; 8: 293–305.

20 Kimchi-Sarfaty C, Oh JM, Kim IW, et al. A "silent" polymorphism in the *MDR1* gene changes substrate specificity. *Science* 2007; 315: 525–28.

21 Hulot JS, Bura A, Villard E, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006; 108: 2244–47.

22 Fontana P, Hulot JS, De Moerloose P, Gaussem P. Influence of *CYP2C19* and *CYP3A4* gene polymorphisms on clopidogrel responsiveness in healthy subjects. *J Thromb Haemost* 2007; 5: 2153–55.

23 Brandt JT, Close SL, Iturria SJ, et al. Common polymorphisms of *CYP2C19* and *CYP2C9* affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J Thromb Haemost* 2007; 5: 2429–36.

24 Gladding P, Webster M, Zeng I, et al. The pharmacogenetics and pharmacodynamics of clopidogrel response: an analysis from the PRINC (plavix response in coronary intervention) trial. *JACC Cardiovasc Interv* 2008; 1: 620–27.

25 Wiviott SD, Antman EM, Gibson CM, et al. Evaluation of prasugrel compared with clopidogrel in patients with acute coronary syndromes: design and rationale for the TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet InhibitioN with prasugrel Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38). *Am Heart J* 2006; 152: 627–35.

26 Zhou SF, Di YM, Chan E, et al. Clinical pharmacogenetics and potential application in personalized medicine. *Curr Drug Metab* 2008; 9: 738–84.

27 Moriya Y, Nakamura T, Horinouchi M, et al. Effects of polymorphisms of *MDR1*, *MRP1*, and *MRP2* genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects. *Biol Pharm Bull* 2002; 25: 1356–59.

28 Nakamura T, Sakaeda T, Horinouchi M, et al. Effect of the mutation (C3435T) at exon 26 of the *MDR1* gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002; 71: 297–303.

29 Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 2000; 97: 3473–78.